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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C12Q 1/68, 1/70, C12N 7/00, 7/01, 7/02, G01N 33/564, C07K 1/00</b>		<b>A1</b>	(11) International Publication Number: <b>WO 98/59074</b> (43) International Publication Date: 30 December 1998 (30.12.98)
(21) International Application Number: <b>PCT/US98/12990</b> (22) International Filing Date: 23 June 1998 (23.06.98)  (30) Priority Data: 60/050,548 23 June 1997 (23.06.97) US 60/057,606 4 September 1997 (04.09.97) US  (71) Applicant: EMORY UNIVERSITY [US/US]; 2009 Ridgewood Drive, Atlanta, GA 30322 (US). (72) Inventors: NOVEMBRE, Francis, J.; 195 Chateau Way, Smyrna, GA 30082 (US). MCCLURE, Harold, M.; 2301 Poplar Springs Drive, Atlanta, GA 30319 (US). (74) Agents: FERBER, Donna, M. et al.; Greenlee, Winner and Sullivan, P.C., Suite 201, 5370 Manhattan Circle, Boulder, CO 80303 (US).			(81) Designated States: AU, CA, CN, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  Published <i>With international search report.</i>
(54) Title: HUMAN IMMUNODEFICIENCY VIRUSES CAUSING AIDS IN A NONHUMAN PRIMATE			
(57) Abstract  An isolated human immunodeficiency virus (HIV) type 1, having the identifying characteristics of HIV isolate JC and assigned AIDS Reagent Program Catalog Number 3523, was isolated from an HIV-infected chimpanzee that developed AIDS. This chimpanzee represents the first known animal model of HIV-1 induced AIDS. The substantially full-length (infectious) nucleotide sequences of the HIV-1 <sub>JC</sub> and of HIV-1 <sub>NC</sub> molecular clones are provided herein. This HIV-1 <sub>JC</sub> and HIV-1 <sub>NC</sub> isolates are useful for the preparation of recombinant, attenuated and subunit vaccines, as well as for the preparation of challenge stocks. It is also used as a diagnostic reagent in screening for the presence of HIV-1 in biological samples.			

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## HUMAN IMMUNODEFICIENCY VIRUSES CAUSING AIDS IN A NONHUMAN PRIMATE

### CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from United States Provisional Patent Application No. 60/050,548, filed June 23, 1997 and from United States Provisional Patent Application No. 60/057,606, filed September 4, 1997.

### ACKNOWLEDGEMENT OF FEDERAL RESEARCH SUPPORT

This invention was made, at least in part, with funding from Public Health Service Grant RR-00165 from the National Center for Research Resources. The United States government may have certain rights in this invention.

### BACKGROUND OF THE INVENTION

This invention concerns unique isolates of human immunodeficiency virus type 1 (HIV-1<sub>IC</sub>) which are highly infectious *in vivo* and produce acquired immune deficiency syndrome (AIDS) in a nonhuman primate.

The human immunodeficiency viruses types 1 and 2 (HIV-1, HIV-2) are retroviruses which have been implicated as the causative agents of acquired immune deficiency syndrome (AIDS) (Barre-Sinoussi et al. [1983] *Science* **220**:868-871). While skepticism about the exact cause of AIDS has arisen (Duesberg [1991] *Proc. Natl. Acad. Sci.* **88**:1575-1579), a large amount of data has accumulated which supports HIV as the biologic agent of this disease. The most convincing evidence includes: mother to child transmission; transmission

via blood transfusion; and transmission via contaminated blood products (Curran et al. [1984] N. Engl. J. Med. 310:69-75). The development of disease in an animal inoculated with HIV-1 would provide confirmatory evidence for the etiology of AIDS. While AIDS-like disease has been recently demonstrated in HIV-2-infected baboons (Barnett et al. [1994] Science 266:642-646), to date, no species, other than humans, has developed AIDS following infection with HIV-1. Thus, the most appropriate animal model for HIV-1 infection currently does not exist. However, an effective alternative animal model has been developed using simian immunodeficiency virus (SIV) infection of macaques. This model has been extremely useful for investigating the pathogenesis of immunodeficiency virus infection and for the testing of vaccines and therapies (Gardner et al. [1994] in *The Retroviruses*, vol. 3 [Levy, ed.] Plenum Press, New York).

Experimental infection of nonhuman primates with human immunodeficiency virus type 1 (HIV-1) has been described for gibbons (Lusso et al. [1988] J. Immunol. 141:2467-2473), pig-tailed macaques (Barre-Sinoussi et al., *supra*), and chimpanzees (Alter et al. [1984] Science 226:549-552; Fultz et al. [1986] J. Virol. 58:116-124). However, the development of disease (AIDS) has not been documented in these animals or in any animal species infected with HIV-1. For many years, chimpanzees have been a major focus in the development of animal models for HIV-1 infection and therapy. The ability to consistently infect chimpanzees with several HIV-1 subtypes and to reisolate the virus over extended periods (Fultz et al., *supra*; Johnson et al. [1993] AIDS Res. Hum. Retroviruses 9:375-378) has made chimpanzees useful for testing vaccine candidates (Berman et al. [1990] Nature 345:622-625; Fultz et al. [1992] Science 256:1687-1690; Girard et al. [1995] J. Virol. 69:6239-6248). However, the lack of disease in HIV-1-infected chimpanzees has raised concern over the relevance of these vaccine studies.

The present invention provides HIV-1 isolates that are infectious to nonhuman primates such as chimpanzees and induce AIDS in inoculated nonhuman primates including, but not limited to, chimpanzees. Further, this invention provides for the first time a proper animal model for HIV-1 infection and for the development of AIDS.

## SUMMARY OF THE INVENTION

A first aspect of the present invention are the isolated human immunodeficiency virus type 1 (HIV-1) isolate having the identifying characteristics of HIV-1 isolate JC (HIV-1<sub>JC</sub>) and assigned AIDS Reagent Program Catalog Number 3523 and the isolate having the identifying characteristics of HIV-1 isolate NC (HIV-1<sub>NC</sub>). These viruses are useful for the preparation of recombinant, attenuated and subunit vaccines, as well as for the preparation of challenge stocks. Each is also useful in screening for the presence of HIV in biological samples.

A second aspect of the invention is a biological sample, e.g., a biological fluid or tissue, containing an HIV-1 having the identifying characteristics of HIV-1<sub>JC</sub> or HIV-1<sub>NC</sub>. In a particular embodiment of the invention, primate blood specimens containing the HIV-1<sub>JC</sub> or the HIV-1<sub>NC</sub> of the present invention were used to cause HIV infection and to induce AIDS in a nonhuman primate, as specifically exemplified by the chimpanzee.

A third aspect of the invention are a biologically pure culture of host cells containing an HIV-1 having the identifying characteristics of HIV-1<sub>JC</sub> and a biologically pure culture of host cells containing an HIV-1 having the identifying characteristics of HIV-1<sub>NC</sub>.

A further aspect of the invention are isolated DNA molecules which produce infectious HIV-1 having the identifying characteristics of HIV-1<sub>JC</sub> or which encode an antigenic fragment thereof or having the identifying characteristics of HIV-1<sub>NC</sub> or encode an antigenic fragment thereof. The substantially full length sequence for infectious molecular clone of HIV-1<sub>JC</sub> is given in SEQ ID NO:11, and the corresponding sequence for the infectious molecular clone of HIV-1<sub>NC</sub> is given in SEQ ID NO:12.

An additional aspect of the invention provides isolated DNA encoding an HIV-1 envelope protein having the amino acid sequence of SEQ ID NO:2. In a particular embodiment, the DNA sequence encoding an HIV-1 envelope protein contains the nucleotide sequence of SEQ ID NO:1.

A further aspect of the invention are a composition comprising an antigenic preparation derived from the HIV-1<sub>IC</sub> of the invention and a composition comprising an antigenic preparation derived from the HIV-1<sub>NC</sub> of the present invention.

An additional aspect of the invention are pharmaceutical compositions comprising an immunogenic amount of an antigenic preparation derived from the HIV-1<sub>IC</sub> of the invention in a pharmaceutically acceptable carrier or HIV-1<sub>NC</sub> in a pharmaceutically acceptable carrier.

A further aspect of the present invention is a kit for detecting the presence of HIV-1 antibodies, comprising an antigenic preparation derived from the foregoing HIV-1<sub>IC</sub> or from HIV-1<sub>NC</sub> as described herein.

Another aspect of the invention is a method of inducing in a subject antibodies to the HIV-1<sub>IC</sub> or to HIV-1<sub>NC</sub> of the invention, comprising the step of administering to the subject an immunogenic amount of an antigenic preparation derived from the HIV-1<sub>IC</sub> or from the HIV-1<sub>NC</sub>. The induced antibodies can be harvested and find use, for example, as hybridization probes in methods for HIV scanning.

A further aspect of the invention is a method of immunizing a subject against infection by HIV, comprising the step of administering to the subject an immunogenic amount of an antigenic preparation derived from the HIV-1<sub>IC</sub> or derived from HIV-1<sub>NC</sub> of the present invention.

It is another aspect of the invention to provide a method for inducing acquired immune deficiency syndrome (AIDS) in a nonhuman primate, comprising the step of administering to said primate an effective amount of an antigenic preparation derived from the HIV-1<sub>IC</sub> and/or HIV-1<sub>NC</sub> of the invention such that said primate develops AIDS. In a specific embodiment of the invention, HIV-1<sub>IC</sub> induced AIDS in chimpanzees, e.g., in a chimpanzee referred to as C499. In a different embodiment of the invention, HIV-1<sub>IC</sub> contained in a biological sample from a chimpanzee having AIDS (C499) induces AIDS in a second chimpanzee and in a different primate species.

It is another aspect of the invention to provide a method for inducing acquired immune deficiency syndrome (AIDS) in a nonhuman primate, comprising the step of administering to said primate an effective amount of an antigenic preparation derived from the HIV-1<sub>NC</sub> of the invention such that said primate develops AIDS. In a specific embodiment of the invention, HIV-1<sub>NC</sub> induced AIDS in chimpanzees, e.g., in a chimpanzee referred to as C534. In a different embodiment of the invention, HIV-1<sub>NC</sub> contained in a biological sample from a chimpanzee having AIDS (C455) induces AIDS in a second chimpanzee and in a different primate species.

A further aspect of the invention provides a biological fluid or tissue sample, obtained from a chimpanzee having HIV-1<sub>JC</sub>-induced AIDS, containing an antigenic HIV fragment for inducing AIDS in a nonhuman primate.

A further aspect of the invention provides a biological fluid or tissue sample, obtained from a chimpanzee having HIV-1<sub>NC</sub>-induced AIDS, containing an antigenic HIV fragment for inducing AIDS in a nonhuman primate.

It is yet another aspect of the invention to provide a method for inducing AIDS in a nonhuman primate, comprising the step of administering to said primate an effective amount of a biological fluid or tissue sample, obtained from a primate having HIV-1<sub>JC</sub>-induced or HIV-1<sub>NC</sub>-induced AIDS, containing an antigenic HIV fragment for inducing AIDS in a nonhuman primate.

The present invention further provides various vaccine formulations containing active immunogenic agents derived from the foregoing HIV-1<sub>JC</sub>, DNA encoding the HIV-1<sub>JC</sub>, and DNA encoding antigenic fragments of the HIV-1<sub>JC</sub>, from the foregoing HIV-1<sub>NC</sub>, DNA encoding the HIV-1<sub>NC</sub>, and DNA encoding antigenic fragments of the HIV-1<sub>NC</sub>. An antigenic fragment contains one or more epitopes which bind antibodies directed to the HIV-1<sub>JC</sub> and/or HIV-1<sub>NC</sub> of the invention.

## BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the CD4+ -cell decline and plasma virus loads in chimpanzee C455 following transfusion with blood from C499. Immediately before and at various times after transfusion, blood was collected from C455 for *in vitro* analyses of CD4+ -cell levels and plasma virus loads. Absolute peripheral CD4+ cells in C455 showed a dramatic decrease by 2 weeks post-transfusion. This rapid decline continued and, by 14 weeks after transfusion, the number of CD4+ cells decreased to 10/ $\mu$ l. These low cell numbers have been maintained to date (42 weeks post-transfusion). Plasma HIV-1 RNA loads in C455 showed high levels of virus present by two weeks after transfusion. Results obtained at the 4-week point suggest very high levels of virus replication in C455. However, HIV-1 levels appeared to be somewhat controlled by 5 to 9 weeks post-transfusion. The cutoff level of  $10^4$  equivalents/ml (---) is the lower limit of the assay.

Fig. 2 illustrates CD4+ -cell decline in chimpanzee C534 following transfusion with blood from C455. Immediately before and at various times after transfusion, blood was collected from C534 for *in vitro* analyses of CD4+ -cell levels. Absolute peripheral CD4+ cells in C534 showed a dramatic decrease by 20 days post-transfusion.

Figure 3 illustrates the strategy used in PCR amplification of subgenomic fragments from HIV-1 and the location and orientation of primers on the viral genome are shown. All the primers were designed from the HIV-1<sub>LA1</sub> nucleotide sequence and their coordinates are described below. PCR amplified fragments were cloned in TA vectors and the corresponding name designations for the recombinant plasmids are shown in bracket. The 5'-LTR-containing *Apa I* fragment amplified from HIV-1<sub>JC</sub> PBMC genomic DNA was subcloned in pJC to generate plasmid pHIV-1<sub>JC16</sub> while the *Apa I*-*Nco I* fragment containing the 5'-LTR region amplified from HIV-1<sub>NC</sub> PBMC genomic DNA was subcloned in pHIV-1<sub>JC16</sub> to generate pHIV-1<sub>NCJC</sub> chimeric plasmid. Plasmid pHIV-1<sub>NC7</sub> was constructed by subcloning the *env*-containing *Nco I*-*Xho I* fragment amplified from HIV-1<sub>NC</sub> genomic DNA to pHIV-1<sub>NCJC</sub> chimeric plasmid. All recombinant plasmids (pHIV-1<sub>JC16</sub>, pHIV-1<sub>NCJC</sub>, and pHIV-1<sub>NC7</sub>) lacked 55 bp at the 5' end of the genome (U3 region) and all of U5 region in the 3' LTR region.



Figures 4A-4F show replication of cloned and uncloned HIV-1 isolates in con-A stimulated and unstimulated chimpanzee PBMC (cPBMC). Stimulated cPBMC infected with (Fig. 4A) HIV-a LAV-1b and SF2 parental strains and the highly cytopathic DH12 isolate, (Fig. 4B) JC (uncloned) and JC16 (cloned) isolates of HIV-1, and (Fig. 4C) NC (uncloned) and NC7 (cloned) isolates of HIV. Unstimulated cPBMC infected with: (Fig. 4D) LAV-1b, SF2, and DH12; (Fig. 4E) JC, JC16; and (Fig. 4F) NC, NC7. Chimpanzee PBMC ( $1.1 \times 10^7$ ) in T-25 cm<sup>2</sup> were infected with 20 ng of either HIV-1<sub>NC</sub> (uncloned), HIV-1<sub>SF2</sub>, HIV-1<sub>LAV-1b</sub>, or HIV-1<sub>DH12</sub> virus and incubated for a total of 17 days at 37°C. Supernatant aliquots were made on 3, 7, 10, 14, and 17 days post infection. Reverse transcriptase (RT) assays were performed as outlined hereinbelow.

Figure 5 shows replication of HIV-1 isolates in chimpanzee monocyte-derived macrophages (MDM). Purified PBMC ( $6 \times 10^6$ /well) were used to obtain MDM. Ten ng of each of virus HIV-1<sub>JC16</sub> (molecular clone), HIV-1<sub>JC</sub> (uncloned), HIV-1<sub>NC7</sub> (molecular clone), HIV-1<sub>NC</sub> (uncloned), HIV-1<sub>SF2</sub>, HIV-1<sub>LAV-1b</sub>, and HIV-1<sub>DH12</sub> was used for infection and on days 7 and 14 post infection supernatants were harvested. The mount of virus in the supernatants was determined using the p24 HIV-1 antigen capture ELISA (Coulter). Supernatants from control uninfected cultures are represented.

#### DETAILED DESCRIPTION OF THE INVENTION

The following definitions are given in order to provide clarity as to the intent or scope of their usage in the specification and claims.

The term HIV-1 isolate JC or HIV-1<sub>JC</sub> or HIV<sub>JC</sub> or HIV-1 having the identifying characteristics of HIV-1<sub>JC</sub> as used herein refers to the particular HIV-1 isolated from a chimpanzee (C499) that developed AIDS 10 years after infection with HIV-1 (Novembre et al. [1997] J. Virol. 71:4086-4091). The DNA sequence of the cloned HIV-1<sub>JC</sub> is given in SEQ ID NO:11.

The term HIV-1 isolate NC or HIV-1<sub>NC</sub> or HIV<sub>NC</sub> or HIV-1 having the identifying characteristics of HIV-1<sub>NC</sub> as used herein refers to the particular HIV-1 isolated from a chimpanzee (C455) that developed AIDS after infection with HIV-1<sub>IC</sub> (Novembre et al. [1997] J. Virol. 71:4086-4091). The DNA sequence of the cloned HIV-1<sub>NC</sub> is given in SEQ ID NO:12.

The term antigenic preparation of HIV-1<sub>IC</sub> or antigenic fragment of HIV-1<sub>IC</sub> as used herein refers to the whole viral particle of the HIV-1<sub>IC</sub> or to a fragment thereof, wherein such fragment encodes at least one epitope or antigenic determinant. The term antigenic preparation of HIV-1<sub>NC</sub> or antigenic fragment of HIV-1<sub>NC</sub> as used herein refers to the whole viral particle of the HIV-1<sub>IC</sub> or to a fragment thereof, wherein such fragment encodes at least one epitope or antigenic determinant.

The term animal or subject as used herein refers to a mammal and, more frequently, to a primate.

The term effective amount as used herein refers to the quantity of active ingredient necessary to effect in an animal a change in a specific biochemical or immunological parameter. For example, in a particular embodiment of this disclosure, an effective amount refers to the amount of HIV-1 administered to a subject such that viral infection and AIDS developed.

The term antigenic amount as used herein refers to the quantity of active antigen necessary to effect an interaction with corresponding antibodies.

The term immunogenic amount as used herein refers to the quantity of active antigen necessary to stimulate the immune system in response to a specific antigen.

The term labeled is used herein to refer to the conjugating or covalent bonding of any suitable detectable group, including enzymes (e.g., horseradish peroxidase,  $\beta$ -glucuronidase, alkaline phosphatase, and  $\beta$ -D-galactosidase), fluorescent labels (e.g., fluorescein, luciferase),

and radiolabels (e.g.,  $^{14}\text{C}$ ,  $^{131}\text{I}$ ,  $^3\text{H}$ ,  $^{32}\text{P}$ , and  $^{35}\text{S}$ ) to the compound being labeled. Techniques for labeling various compounds, including proteins, peptides and antibodies are well known. See, e.g., Morrison, *Methods in Enzymology* 32B, 103 (1974); Syvanen et al., *J. Biol. Chem.* 284, 3762 [1973]; Bolton and Hunter, *Biochem. J.* 133, 529 [1973].

The viruses of the present invention resulted from the development of AIDS in a first chimpanzee infected with HIV-1 for over 10 years and by the rapid development of immunosuppression in a second chimpanzee transfused with blood from the first chimpanzee. To investigate the ability of HIV-1 to induce AIDS in non-human primates, a cohort of 12 chimpanzees was inoculated with several strains of HIV-1 at the Yerkes Center in the mid-1980s. A member of this cohort, C499, was described as part of a previously reported superinfection study (Fultz et al. [1987] *J. Virol.* 61:4026-4029) and was inoculated on three separate occasions with three different HIV-1 isolates: HIV-1<sub>SF2</sub> in 1985, HIV-1<sub>LA1</sub> in 1986, and HIV-1<sub>NDK</sub> in 1987. The first inoculation resulted in infection, as determined by positive virus isolation and persistent HIV-1-specific antibody response. Clinically, the animal remained healthy except for the development of thrombocytopenia and lymphopenia in 1988, which resolved without treatment (Fultz et al. [1991] *J. Infect. Dis.* 163:441-447). (All HIV-infected chimpanzees are maintained in Biosafety level 3 isolation facilities.)

In 1993, after the resumption of yearly monitoring (monitoring of all HIV-1-infected chimpanzees at the Yerkes Center was suspended from March 1990 to May 1993), a decrease in the levels of platelets and CD4<sup>+</sup> cells in C499 was observed (24,000 and 390/ $\mu\text{l}$ , respectively) (Table 1). Thrombocytopenia and CD4<sup>+</sup>-cell lymphopenia were persistent in this animal from this point onward. In addition, C499 displayed other significant clinical signs of disease. Beginning in March 1995, C499 developed chronic, intermittent diarrhea for which no enteric pathogens were identified and which was not resolved with antibiotic treatments. In September 1995, this animal developed acute fulminant diarrhea which was associated with large numbers of *Blastocystis hominis* and *Balantidium coli*. At this time, CD4<sup>+</sup> cells decreased to extremely low levels (minimum of 10/ $\mu\text{l}$ :2% of total T cells) (Table 1), indicative of severe immunosuppression. Similarly, declines were observed in the levels of total lymphocytes and CD8<sup>+</sup> cells (Table 1). Treatment with fluid replacement and

antimicrobial and antiprotozoal therapy (doxycycline, ceftriaxone, enrofloxacin, gentamicin, and albendazole) resulted in the resolution of acute diarrhea within five days.

Because this was the first chimpanzee to develop AIDS, treatment with antiretroviral therapies was not administered in order to more fully characterize virological, immunological, and pathological parameters in this animal. As the acute diarrhea was resolved with treatment, CD4<sup>+</sup>-cell levels rose to a maximum of 180/ $\mu$ l (4% of total T cells) but declined again. Subsequently, chronic, intermittent diarrhea resumed and continued unresolved. During this period, C499 exhibited no lymphadenopathy or wasting. However, beginning in the latter part of 1995 and extending into 1996, C499 developed progressive nonregenerative anemia (hematocrit levels of 37.5% in December 1995 and 27.2% in January 1996, with respective hemoglobin values of 12.1 and 8.4 g/dl; reticulocyte counts were 0.0% since November 1995). Due to progressive hematologic abnormalities, chronic diarrhea, and continued immunosuppression, the animal was euthanized in February 1996.

Concomitant with the decrease in CD4<sup>+</sup> cells was an increase in HIV-1 loads in plasma (Table 1). The increase in the level of the virus was detected in plasma samples dating from May 1993, when the CD4<sup>+</sup>-cell decline was first noted, but not before the suspension of monitoring in 1990. These levels are significantly higher than those for five other chimpanzees at the Yerkes Center which received cell-free or cell-associated HIV-1<sub>LAI</sub> or HIV-1<sub>sf2</sub> inoculations (all have undetectable plasma viral RNA levels and are not immunosuppressed). These results suggest that pathogenic effects began to occur sometime between 1990, when CD4<sup>+</sup>-cell counts were at normal levels and viral loads were undetectable, and 1993, when alterations in the number of CD4<sup>+</sup> cells and significant virus loads were present.

The ability to isolate virus from C499 varied since C499's first exposure to HIV-1. In general, early after inoculation, HIV was easily isolated from the peripheral blood mononuclear cells (PBMC) of this animal; however, after several months, virus could no longer be isolated. From August 1988 until the suspension of monitoring in 1990, the virus was consistently isolated from C499 on a monthly basis. Subsequently, after the resumption

of monitoring in mid-1993, HIV-1 continued to be easily isolated from this animal. Quantitative titration of PBMC viral load following the development of acute diarrhea in C499 in September 1995 until the time of euthanasia revealed that  $10^4$  to  $10^5$  PBMC was consistently required for virus isolation. The immune response of C499 to HIV-1 infection was very strong up to the time of euthanasia. HIV-1 antibody endpoint titers (HIV-1 whole-virus enzyme-linked immunosorbent assay, Genetic Systems, Redmond, WA) ranged from 51,200 to 204,800 since 1993. Because of the deteriorating condition of this animal and because of the severe decline in CD4<sup>+</sup> cells, it was hypothesized that the HIV-1 present in this animal had evolved to become more cytopathic for chimpanzee CD4<sup>+</sup> cells. Cocultivation of PBMC derived from C499 (obtained at the time of acute diarrhea) with uninfected chimpanzee PBMC (cPBMC) resulted in the isolation of a virus (HIV-1<sub>JC</sub>) which induced syncytium formation in chimpanzee cells (Fig. 1A-D). This characteristic has been previously described for only three HIV-1 isolates (Ghosh et al. [1993] *Virology* 194:858-864; Schuitemaker et al. [1993] *J. Infect. Dis.* 168:1140-1147; Shibata et al. [1995] *J. Virol.* 69:4453-4462), none of which were used for inoculation of C499.

The virus strain derived from C499 (HIV-1<sub>JC</sub>) and other HIV-1 isolates (HIV-1<sub>LAI</sub> and HIV-1<sub>SF2</sub>) were tested for the ability to induce syncytium formation in cPBMC. Virus stocks were prepared in cPBMC (HIV-1<sub>JC</sub>) or in human PBMC (HIV-1<sub>LAI</sub> and HIV-1<sub>SF2</sub>). Cells were incubated with virus overnight and were then washed. Cultures were examined daily for evidence of cytopathic effects. cPBMC four days after infection with an HIV-1 isolate (HIV-1<sub>JC</sub>) from C499 showed beginnings of syncytium formation and separated syncytia. cPBMC four days after infection with HIV-1<sub>LAI</sub> lacked of syncytium formation, and normal cell clusters were present. cPBMC four days after infection with HIV-1<sub>SF2</sub> also showed no syncytium formation. The only virus to induce significant cytopathic effects in cPBMC was HIV-1<sub>JC</sub>. All cultures were examined for 14 days following infection. All cultures, regardless of the virus used, became positive for virus replication by seven days postinfection. Thus, it is concluded that genetic changes which confer the ability to induce syncytium formation occurred in the virus present in C499.

To confirm that the virus present in C499 was different from the viruses used for inoculation, DNA prepared from HIV-1<sub>JC</sub>-infected cPBMc was used as a template in typical PCR assays with primers (forward, no. 384: 5'CCCTTCGAAGAGGATATAATCAGTTTATGGGATCAAAGC3' [SEQ ID NO:9]; reverse, no. 383: 5'CCCTTCGAACCTCTTCTTCTGCTAGACTGCCATT3' [SEQ ID NO:10]) designed to amplify a 507-bp fragment of the *env* gene containing the V1 and V2 regions. Genetic analysis of 16 HIV-1<sub>JC</sub> V1-V2 clones obtained by ligation of the amplification products with the vector pGEM7ZF (Promega, Madison, WI) showed amino acid homologies of 80 to 84% with HIV-1<sub>LAI</sub>, 73 to 80% with HIV-1<sub>SF2</sub>, and 63- to 68% with HIV-1<sub>NDK</sub>. Thus, there appears to be considerable divergence between the virus present in C499 at the time of acute disease and the viruses used to inoculate this animal. This divergence is further illustrated in Fig. Tables 3A-3C, which show amino acid alignments of five HIV-1<sub>JC</sub> clones, HIV-1<sub>NDK</sub>, HIV-1<sub>LAI</sub>, and HIV-1<sub>SF2</sub>. Comparative analyses between the 16 HIV-1<sub>JC</sub> clones showed that amino acid homologies ranged from 81 to 96%, with no clones being identical. These results suggest that the virus population in C499 consisted of a large quasispecies. Furthermore, the data, when combined with the *in vitro* analyses described above, indicate that the virus adapted after years of replication and mutation, becoming more pathogenic for the chimpanzee. While no evidence of recombination is evident from analyses performed in this small area, the possibility of recombination cannot be ruled out for other portions of the HIV-1<sub>JC</sub> genome.

Tables 2A-2C illustrate amino acid alignment of V1-V2 clones obtained from HIV-1<sub>JC</sub>-infected cPBMc and prototypes HIV-1<sub>LAI</sub> and HIV-1<sub>SF2</sub>, and HIV-1<sub>NDK</sub>. *env* clones of HIV-1<sub>JC</sub> encompassing the V1-V2 region were sequenced by the dideoxy chain termination method (Sequenase; Amersham Life Science, Arlington Heights, IL). With the Intelligenetics Suite of programs (Intelligenetics, Beaverton, OR), sequences of HIV-1<sub>JC</sub> *env* fragments were used to derive corresponding amino acid sequences. Deduced amino acid sequences from five of these clones (HIV<sub>JC</sub>10, HIV<sub>JC</sub>17, HIV<sub>JC</sub>45, HIV<sub>JC</sub>48, and HIV<sub>JC</sub>55) were then aligned with the corresponding region in HIV-1<sub>LAI</sub>, HIV-1<sub>SF2</sub>, and HIV-1<sub>NDK</sub> isolates. Amino acid sequences of HIV-1<sub>LAI</sub>, HIV-1<sub>SF2</sub> and HIV-1<sub>NDK</sub> were obtained from the Human Retroviruses and AIDS Database.

To investigate additional pathogenic effects of HIV-1 infection on C499, tissue samples obtained by biopsy during the acute diarrheal stage (September 1995) and at necropsy were subjected to histopathological analyses. First, examination of a peripheral lymph node (obtained from C499 in September 1995) revealed marked lymphoid depletion within the cortical area, with a few follicles remaining, as compared with a lymph node from an age-matched, uninfected chimpanzee, which lacked follicular development and had a very cellular cortex. To detect virus expression in tissues, *in situ* hybridization experiments with digoxigenin-labeled riboprobes encompassing the entire HIV-1 genome (derived from the HIV-1 BH10 molecular clone (Hahn et al. [1984] Nature 312:166-169) were used to probe formalin-fixed lymph node sections. Detection of bound probes was performed with a sheep anti-digoxigenin-alkaline phosphatase-labeled Fab monoclonal antibody and with Nitro Blue Tetrazolium-5-bromo-4-chloro-3-indolylphosphate toluidinium as the substrate chromogen, according to previously described methods (Hirsch et al. [1995] J. Virol. 69:955-967). *In situ* hybridization studies of C499's lymph node demonstrated the presence of HIV-1 RNA in follicular trapping patterns, with few positively staining cells. These findings are similar to changes observed in HIV-1-infected persons developing AIDS (Pantaleo et al. [1995] N. Engl. J. Med. 332:209-216). Control samples, which included sense riboprobes and lymph nodes from uninfected chimpanzees, did not show any staining. Additional lymph nodes obtained from C499 at necropsy showed a similar depleted pattern. However, a few lymph nodes were not as depleted but did contain multinucleated giant cells which stained positive for the HIV-1 p24 antigen. These giant cells are often found in the lymph nodes of simian immunodeficiency virus (SIV)-infected macaques and are occasionally found in the lymph nodes in HIV-1-infected persons.

Finally, histopathologic analysis of intestinal tissue from C499 revealed pathologic changes in the ileum, with significant blunting of the villi and intense infiltration of mononuclear cells and plasma cells. Examination of the intestinal mucosa at higher magnification revealed extensive infection with *Cryptosporidium*, which was present throughout the small intestine, lining the apical surfaces of intestinal epithelial cells. This organism is an AIDS-defining opportunistic pathogen (Centers for Disease Control and Prevention. 1992. 1993 revised classification system for HIV infection and expanded

surveillance case definition for AIDS among adolescents and adults. Morbid. Mortal. Weekly Rep. 41:1-19) and probably accounted for the chronic intermittent diarrhea and intestinal pathology in C499.

Most tissues obtained from C499 following euthanasia appeared grossly normal, with no lymphadenopathy or splenomegaly. However, the spleen showed moderate congestion and lacked follicle development. Bone marrow specimens obtained at euthanasia showed some functional impairment in CFU granulocyte macrophage and CFU formation, although levels of CD4<sup>+</sup> cells were not altered (Villinger et al. [1997] J. Med. Primatol 26:181-189. Virus was isolated from all lymphoid organs including inguinal, axillary, and mesenteric lymph nodes as well as from the spleen and thymus. Virus was also isolated from the kidney and liver but not from the brain or cerebrospinal fluid.

At the time of acute diarrhea in C499, a blood transfusion was performed to determine the effects of passage of virus from this animal to an uninfected chimpanzee. Forty milliliters of blood obtained by venipuncture from C499 was immediately transfused intravenously into C455. This chimpanzee, which was bred in captivity, was seropositive for Epstein-Barr virus and cytomegalovirus and was seronegative and PCR negative for HIV prior to the transfusion. Results of titration analysis of PBMC and plasma from C499 show that in the 40 ml of blood,  $1 \times 10^4$  50% tissue culture infective doses (TCID<sub>50</sub>) of virus was in PBMC and  $2 \times 10^4$  TCID<sub>50</sub> of virus was in plasma. Thus, C455 received a total of  $3 \times 10^4$  TCID<sub>50</sub> of virus. Analysis of peripheral CD4<sup>+</sup> cell levels in C455 revealed a precipitous decline beginning by 2 weeks after transfusion, when absolute numbers of CD4<sup>+</sup> cells decreased from 1,240 to 320 cells/ $\mu$ l (Fig. 1). This decline continued, reaching a minimum value of 10 cells/ $\mu$ l (1% of total T cells) by 14 weeks post-transfusion. Since this time, the total percentage of T cells that the CD4<sup>+</sup> population encompasses has remained constant at 1%, with only a slight rise in the absolute number of CD4<sup>+</sup> cells (20 cells/ $\mu$ l at the latest time point). Levels of peripheral CD8<sup>+</sup> cells showed an initial decline from 1,590 to 880 cells/ $\mu$ l in the first two weeks after transfusion. However, these levels quickly rebounded to 2,010 CD8<sup>+</sup> cells/ $\mu$ l by 8 weeks after transfusion. Since this time, the number of CD8<sup>+</sup> cells in circulation has been maintained between 550 and 4,320/ $\mu$ l, with the level in most recent sample being 840 cells/ $\mu$ l.



Because CD4<sup>+</sup>-cell levels declined so rapidly, the level of virus present in C455 was investigated by quantitation of plasma HIV-1 RNA with the Chiron B-DNA assay (Fig. 1). Two weeks after transfusion, plasma viral loads were  $2 \times 10^7$  RNA equivalents/ml and reached maximum levels by 4 weeks post-transfusion ( $6.2 \times 10^7$  RNA equivalents/ml). Following a decline (which corresponded with the development of anti-HIV-1 specific antibody), plasma HIV-1 levels have been maintained at  $\sim 1.1 \times 10^5$  RNA equivalents/ml. Virus has been easily isolated from C455 at all times post-transfusion. Quantitative coculture of PBMC from C455 has shown that early after transfusion, virus could be isolated from as few as  $10^2$  PBMC, while at more recent time points, up to  $10^5$  to  $10^6$  cells was required for virus isolation. *In vitro* analysis of virus isolated from this animal at several time points has shown that the ability to induce syncytium formation in cPBMC has been retained, further implicating this virus in the pathogenesis of CD4<sup>+</sup>-cell decline. Antibody responses to HIV-1 have been moderate in C455. Enzyme-linked immunosorbent assay titers have been maintained between 1,600 and 6,400 since four weeks post-transfusion. Clinically, C455 has appeared normal, except for episodic incidences of a rash on the chest and in the scrotum area. In addition, the animal has experienced no weight loss, lymphadenopathy, or anemia.

This invention presents an HIV-1 infected chimpanzee that developed AIDS as defined by the Centers for Disease Control and Prevention classification system (CDCP 1992, *supra*). Progression of clinical disease (anemia, thrombocytopenia, and chronic diarrhea) in this animal was associated with several key findings, including the following: (i) the presence of a virus which is cytopathic for cPBMC *in vitro* and *in vivo* and is genetically distinct from those used for inoculation, (ii) an increase in viral load; (iii) CD4<sup>+</sup>-cell depletion, (iv) lymph node depletion, and (v) the presence of *Cryptosporidium* organisms in the intestine. It appears that the critical change(s) associated with clinical progression may have developed during the period in which C499 was not monitored (mid-1990 through mid-1993). The precipitous CD4<sup>+</sup>-cell decline concomitant with high viral loads displayed in C455, transfused with blood from C499, suggest that the HIV present in C499 has evolved to become more pathogenic for chimpanzees. The increased pathogenicity of a lentivirus after passage into a new host has been previously observed with the adaptation of SIV from sooty

mangabeys to pig-tailed macaques, resulting in the development of the acutely lethal strain SIVsmmPBj14 (Fultz et al. [1989] AIDS Res. Hum. Retroviruses 5:397-409).

Forty milliliters of blood obtained by venipuncture from C455 was immediately transfused intravenously into C534. This chimpanzee, which was bred in captivity, was seropositive for Epstein-Barr virus and cytomegalovirus and was seronegative and PCR negative for HIV prior to the transfusion. Results of titration analysis of PBMC and plasma from C455 show that in the 40 ml of blood,  $1 \times 10^4$  50% tissue culture infective doses (TCID<sub>50</sub>) of virus was in PBMC and  $2 \times 10^4$  TCID<sub>50</sub> of virus was in plasma. Thus, C534 received a total of  $3 \times 10^4$  TCID<sub>50</sub> of virus. Analysis of peripheral CD4<sup>+</sup> cell levels in C534 revealed a precipitous decline beginning by one week after transfusion, when absolute numbers of CD4<sup>+</sup> cells decreased from approximately 1,450 to 700 cells/ $\mu$ l (Fig. 2). The total percentage of T cells that the CD4<sup>+</sup> population encompasses has remained constant at approximately 1%, with only a slight rise in the absolute number of CD4<sup>+</sup> cells (20 cells/ $\mu$ l at the latest time point). Levels of peripheral CD8<sup>+</sup> cells showed an initial decline in the first two weeks after transfusion. However, these levels quickly rebounded after transfusion.

Table 3 shows the antibody response in chimpanzee C534 following transfusion with blood from C455. Immediately before and at various times after transfusion, blood was collected from C534 for *in vitro* analyses of antibody response. Plasma HIV-1 RNA loads in C534 showed high levels of virus present by two weeks after transfusion. Results obtained at the 3-week point indicate very high levels of virus replication in C455. The cutoff level of  $10^4$  equivalents/ml is the lower limit of the assay.

Because CD4<sup>+</sup>-cell levels declined so rapidly, the level of virus present in C534 was investigated by quantitation of plasma HIV-1 antibody response (Table 4). One week after transfusion, the plasma antibody titer was still 0. At two weeks after transfusion, the antibody titer rose to 400 and remained at 1600 at three and four weeks post-transfusion. Virus has been easily isolated from C455 at all times after transfusion. Quantitative coculture of PBMC from C534 has shown that early after transfusion, virus could be isolated from as few as  $10^2$  PBMC, while at more recent time points, up to  $10^5$  to  $10^6$  cells was required for virus

isolation. *In vitro* analysis of virus isolated from this animal at several time points has shown that the ability to induce syncytium formation in cPBMC has been retained, further implicating this virus in the pathogenesis of CD4<sup>+</sup>-cell decline. Antibody responses to HIV-1 have been moderate in C534. Enzyme-linked immunosorbent assay titers have been maintained between 1,600 and 6,400 since four weeks post-transfusion. Clinically, C534 has appeared normal, except for episodic incidences of a rash on the chest and in the scrotum area. In addition, the animal has experienced no weight loss, lymphadenopathy, or anemia (normal hematocrit levels).

This invention presents HIV-1 isolates NC and JC which infected chimpanzees such that the HIV-1 infected chimpanzees develop AIDS as defined by the Centers for Disease Control and Prevention classification system (CDCP 1992, *supra*). Progression of clinical disease (anemia, thrombocytopenia, and chronic diarrhea) in this animal is associated with several key findings, including the following: (i) the presence of a virus which is cytopathic for cPBMC *in vitro* and *in vivo* and is genetically distinct from that used for inoculation, (ii) an increase in viral load; (iii) CD4<sup>+</sup>-cell depletion, (iv) lymph node depletion, and (v) the presence of *Cryptosporidium* organisms in the intestine. It is expected that the critical change(s) associated with clinical progression develop during the period spanning approximately 3 to 5 years after HIV-1 infection. The precipitous CD4<sup>+</sup>-cell decline concomitant with high viral loads displayed in C534, transfused with blood from C455, indicates that the HIV present in C455 remains pathogenic for chimpanzees.

In the past, relevance of the HIV-1-infected chimpanzee as a model for vaccine evaluation was questioned due to lack of disease development. The lack of an animal model which supports pathogenic HIV-1 infection has been a continuing problem for vaccine development. Although the time of progression to disease (>10 years), the currently limited numbers of animals available for use, and the overall high costs associated with working with chimpanzees are deterrents to their widespread use in AIDS research, the potential usefulness of this model cannot be disregarded. The development of AIDS in C499, the fact that additional HIV-infected chimpanzees have depressed CD4<sup>+</sup>-cell counts (<500/ $\mu$ l) and thrombocytopenia, and the rapid progression of the CD4<sup>+</sup>-cell decline in C455 support the

role that this animal model provides in AIDS-related studies. The adaptation of HIV-1 from long-term chimpanzee infection to a pathogenic form provides a critical link for the adaptation of HIV-1 to growth in more readily available nonhuman primate species. The instant invention further contemplates the growth of HIV-1<sub>JC</sub> isolated from C499 and/or HIV-1<sub>NC</sub> from C455 in chimpanzees as well as in pig-tailed or rhesus macaques. In addition, the present invention provides for continued biological and genetic characterization of HIV-1<sub>JC</sub> and HIV-1<sub>NC</sub> with further key insights into the pathogenesis of HIV-1 infection in humans and chimpanzees, for example, for the development of drugs and vaccines for the treatment and prevention of AIDS.

As described above, chimpanzee C499 was initially infected with HIV-1<sub>SF2</sub> in 1985 [Fultz et al. (1986) *J. Virol.* 58, 116-124] and later inoculated with HIV-1<sub>LAV-1b</sub> and HIV-1<sub>NDK</sub> in 1986 and 1987 respectively. Superinfection with HIV-1<sub>LAV-1b</sub> (but not HIV-1<sub>NDK</sub>) was demonstrated by restriction enzyme analysis of PBMC genomic DNA. At the time of disease development in C499, a virus isolate, termed HIV-1<sub>JC</sub>, was obtained by co-culture of C499 PBMC with normal cPBMC. At that time, sequence analysis of the V1-V2 region of env suggested that HIV-1<sub>JC</sub> was most closely related to HIV-1<sub>LAV</sub>. Also at the time of disease development, blood from C499 was transfused into an uninfected chimpanzee (C455) which resulted in a dramatic decline of CD4+ cells by 2 weeks post transfusion. The depressed CD4+ cell count is still maintained to date in this animal. One month post transfusion 50 µl of plasma from C455 was used for in vitro infection of normal chimpanzee PBMC and the resultant virus was designated HIV-1<sub>NC</sub>.

To perform a more thorough analysis of the genetic makeup of the HIV-1<sub>JC</sub> and HIV-1<sub>NC</sub> viruses, we constructed substantially full-length, infectious molecular clones as described in the Examples and in Figure 3. Both clones lacked 55 nucleotides at the 5' end (5' LTR, U3 region) and all of the U5 region in the 3' LTR. Several clones (representing both HIV-1<sub>JC</sub> and HIV-1<sub>NC</sub>) which appeared to be the correct size were tested for biological activity by transfection of CEMx174 cells. Supernatants from transfected cells were used in RT assays to monitor virus production. Two clones, one from each group (HIV-1<sub>JC</sub> [JC16] and HIV-1<sub>NC</sub> [NC7]), were positive by RT and also showed massive syncytia formation (2 to 3 days post

transfection), similar to that observed with uncloned virus. To prepare stock viruses for use in *in vitro* assays, 293 cells were transfected with molecularly cloned DNAs followed by amplification with cPBM as outlined hereinbelow.

The complete nucleotide sequences of JC16 and NC7 were determined as described in Example 7, and the sequences are given in SEQ ID NO:11 and SEQ ID NO:12, respectively. The genomes were determined to be 9193 nt (JC16) and 9196 nt (NC7) in length and contained open reading (ORFs) for all HIV-1-specific structural, regulatory and accessory genes. Alignment of JC16 and NC7 DNA sequences revealed that the two genomes were very similar, but contained a number of nucleotide changes spread throughout the genome.

compared with the parental inoculating viruses, and with gaps introduced to optimize alignment and treated as mismatches, the LTR sequences of JC16 and NC7 had percent nucleotide identities of 92.1% (LAV), 93.9% (SF2), and 89.1% (NDK)—lower than that of the most divergent region between NC7 and JC16 was the V5 region of the *env* gene. JC16 contained a 6 bp deletion in the *gag* gene relative to NC7 while NC7 had 3 bp deletion in the *env* gene region relative to JC16. In the LTR region there was a 98.7% nucleotide identity between JC16 and NC7 with all the changes being localized to the U5 region (Table 4).

Comparative analyses were performed between JC16, NC7, and the parental inoculating viruses, SF2, LAV, and NDK (The sequence of LAV-1b has not yet been determined). When observed upon direct comparison of JC16 and NC7 (Table 3). Most of the host/virus transcription binding factor sequences (sites for AP-1, NF-AT, NF-kB and Sp-1) and the TAR CORE and the Lys-tRNA sites were conserved (or had single point mutations) between the parental (SF2 and LAV strains) and progeny viruses. However, there were 3 point mutations unique to JC16 and NC7 at the NRF/NRE binding site.

The deduced amino acid sequences for all proteins of HIV-1<sub>JC</sub> and HIV-1<sub>NC</sub> were generated using the Intelligenetics suite of programs. Using the Lasergene program (DNASTAR Inc., Madison, WI), multiple alignments of all proteins were constructed to examine similarities between HIV-1<sub>NC</sub>, HIV-1<sub>JC</sub> and the parental viruses (Table 4). Based upon percent homology calculations, Gag, Pol, Vif, Tat, Rev, Env, and Vpu of JC16 and NC7

were most closely related to LAV, with vpr and Nef being most closely related to SF2. In no case was it apparent that the NDK isolate was the origin of a protein sequence. While most changes involved amino acid point mutations, several proteins of JC16 and NC7 contained amino acid insertions or deletions relative to the parental inoculating strains. A closer analysis of amino acid alignments revealed that the percent homologies could be misleading with regards to the origin of the protein. For example, in Tat, JC16 and NC7 were more homologous to LAV than to SF2 (based upon percent homologies). However, the JC16 and NC7 Tat proteins contained 15 amino acid deletions with respect to LAV--similar to that present in the SF2 isolate. Similar findings were observed in Gag and Pol.

Table 5 shows amino acid alignments of Gag, Nef, and Env proteins from JC16, NC7 and the inoculating viruses, LAV, SF2, and NDK. The deduced amino acid sequences for the Gag (a), Nef (b), and Env proteins of HIV-1 were determined using the Intelligenetics Suite of programs (Intelligenetics, Beaverton, OR) and the CLUSTAL method (DNASTAR Inc., Madison, WI) was used for the alignment of proteins. The virus isolates are shown by the designations at the beginning of every line as LAI (HIV-1<sub>LAV</sub>), JC16 (HIV-1<sub>JC16</sub>), NC7 (HIV-1<sub>NC7</sub>), and SF2 (HIV-1<sub>SF2</sub>). Dashes (-) denote amino acid deletion while dots (.) denote amino acid identity. The numbers after the amino acid sequence on the right show the position of the right-most amino acid in the line. The functional domains on Gag (a) and Nef (b) are indicated. MBD (a) refers to membrane-binding domain while MTD (b) refers to membrane-targeting domain. The hypervariable regions in the Env glycoprotein (c) are shown as V1 to V5 and the CD4 binding domain is shown above the sequence. Gp120 (SU) is the outer surface membrane Env glycoprotein. The NDK Env, LAI Env, and SFS Env protein sequences are given in SEQ ID Nos: 3, 4 and 5, respectively. The JC16 and NC7 Env protein sequences are given in SEQ ID Nos: 2 and 23, respectively. There were 7 (JC16 and LAI sequences) and 5 (NC7 sequence) amino acid deletions in the matrix protein (p17) relative to SF2 isolate sequence. At the C-terminal end of Gag polyprotein, the progeny viruses and SF2 virus had 12 amino acid deletions in p6 protein relative to LAV virus sequence (Table 5). However, the capsid (p24) and nucleocapsid (p7) proteins were generally well conserved including the cysteine residues within the zinc-finger domains. Point mutations unique to JC16 and NC7 were present in p17, p24, p7 and p6 peptides. The Lck binding domain

(proline-rich region) within Nef was well conserved with only one point mutation in LAI (Table 5). However, there were 4 amino acid insertions in the SF2 sequence relative to the other viruses at the N-terminal portion of Nef. Sequence analysis of eight other non-infectious clones (4 from JC and 4 from NC) confirmed observations made for the Gag and Nef deletions suggesting that these characteristics are a general property of the viruses obtained from C499 and C455.

The vast majority of point mutations, deletions, and insertions in these clones, relative to the parental viruses, were found in the env glycoprotein region (Tables 4 and 5). Alignment of the Env proteins revealed that all the cysteine residues resident in the protein were conserved between the viruses. There were a total of 30 predicted N-linked glycosylation sequences (Asn-X-Thr or Asn-X-Ser) for the parental strains compared to 27 (NC7) and 29 (JC16) for the progeny viruses. Most of the glycosylation sites reside in the SU portion of Env with HIV-1<sub>SF2</sub> containing the highest number and HIV-1 isolate NC the least (25 for SF2, 24 for LAI and JC, and 23 for NC). Glycosylation of glycoproteins has been shown to influence the immune response toward virus infection. As expected, the gp120 (SU) glycoprotein contained the highest number of mutations. The V1-V2, V3, V4, and V5 hypervariable regions contained 10, 8, 8, and 3 point mutations respectively specific to JC16 and NC7. The V1-V2 region of JC16 and NC7 also contained insertions relative to the other viruses, resulting in amino acid lengths of 72 (progeny viruses), 69 (LAV), 70 (SF2), and 61 (NDK). There were multiple amino acid deletions in the V4 regions and single amino acid insertions in V5 regions of JC16 and NC7 relative to the parental strains. Both the CD4-binding domain and the proteolytic cleavage site (REKR) at the SU/TM junction were perfectly conserved.

The V3 region was the most interesting of the hypervariable regions. While the parental strains contained only 9 basic amino acids (Arg, Lys, and His positively charged residues) JC16 and NC7 HIV-1 isolates had 12 basic and 2 negatively charged residues (Asp and Glu). This gave the progeny viruses a net positive charge of 10 in the entire V3 region and an overall positive charge of +1 (LAV) or +2 (SF2) compared to the parental

strains. Eight of the 10 resultant positive charges for JC and NC isolates are located between residues 10 and 27 of V3 (Fig. 3) compared with 5 of 9 (LAV) and 4 of 9 (SF2) in the same region. At least within this region, JC and NC isolates seem to have a relatively high net positive charge of 4 (relative to SF2) and 3 (relative to LAV). Other researchers have shown that changes in basic amino acids in the middle portion of V3 loop (residues 10 to 27 in Table 5) can alter the syncytium-inducing properties and phenotype of the virus [Bhattacharyya et al. (1996) *AIDS Res. Hum. Retroviruses* 12, 83-90; De Wolf et al. (1994) *AIDS Res. Hum. Retroviruses* 10, 1387-1400; Okada et al. (1994) *AIDS Res. Hum. Retroviruses* 10, 803-811].

To study the biological activities of the cloned and uncloned viruses derived from C499 and C455, we conducted in vitro replication studies. Figures 4A, 4B and 4C shows the results of replication studies in mitogen-stimulated cPBMC. Two of the three viruses used for inoculation of C499, SF2 and LAV-1b, were able to replicate in stimulated cPBMC, albeit with different kinetics (Fig. 4A). The SF2 isolate grew very slowly and to low titers in cPBMC. In contrast, the LAV-1b isolate grew very well and with rapid kinetics, with a high titer of virus already present by day 7 post infection. The SF2 isolate was unable to induce detectable syncytium formation in cPBMC. The LAV-1b isolate, under these conditions, induced very few syncytia, in contrast to previously reported results [Watanabe et al. (1991) *J. Virol.* 65, 3344-3348]. Included in these analyses was the DH12 isolate of HIV-1 [Shibata et al. (1995) *J. Virol.* 69, 4453-4462]. This primary isolate from a human has been shown to be highly cytopathic for cPBMC. While this virus quickly established infection in the stimulated cell population, it did not grow to high titers. The numerous syncytia formed infection with DH12 could account for the lack of growth observed. The uncloned and cloned viruses of JC (Fig. 4B) and NC (Fig. 4C) replicated to levels comparable to those of LAV-1b for the same period of time. The rates of replication for cloned and uncloned viruses were indistinguishable.

The ability of HIV-1 virus isolates to replicate in unstimulated cPBMC was similarly evaluated. Results of this assay (Fig. 4D, 4E and 4F) showed that only the NC (cloned and uncloned) and the LAV-1b isolates of HIV-1 were capable of significant replication in unstimulated cPBMC. Replication rates for these viruses were less than 10% those observed



in stimulated cPBMC. Additionally, the kinetics of virus production in unstimulated cells was much slower than that observed in stimulated cPBMC. Interestingly, JC (cloned and uncloned) isolates of HIV-1 failed to replicate in cPBMC, reflecting an inherent biological difference between the JC and NC viruses. While the DH12 isolate was able to replicate in unstimulated cells, the levels of virus achieved were much less than that of the other viruses. The SF2 isolate was unable to replicate in unstimulated PBMC. Virus recovered from the molecular clones displayed the intrinsic replicative properties exhibited by the viruses they were derived from.

Reports of HIV-1 isolates able to replicate in chimpanzee macrophages has been controversial. To investigate the ability of these viruses and their respective clones to replicate in macrophages, we conducted in vitro assays using purified chimpanzee monocyte-derived macrophages (MDM). Figure 5 shows virus production in macrophages at 7 and 14 days post infection. Both the HIV-1 JC and NC virus isolates (cloned and uncloned) replicated in MDM as determined by the levels of p24 antigen produced. However, the amount of virus produced by the HIV-1<sub>JC16</sub> molecular clone at 14 days post infection was twice that produced by the HIV-1<sub>JC</sub>. The titers for cloned and uncloned HIV-1<sub>NC</sub> viruses were comparable for the same period of time. Among the viruses that were tropic for MDM, HIV-1<sub>JC</sub> (uncloned) produced the least amount of virus. HIV-1<sub>LAV-1b</sub> and HIV-1<sub>DH12</sub> also infected MDM and produced virus which is consistent with previous observations made by Gendelman et al. [Gendelman et al. (1991) *J. Virol.* 65, 3853-3863] and Shibata et al. [Shibata et al. (1995) *J. Virol.* 69, 4453-4462] respectively. Unlike HIV-1<sub>LAV-1b</sub>, the other parental virus, HIV-1<sub>SF2</sub>, did not replicate in MDM.

Because the cloned viruses displayed such similar in vitro biologic activities as the uncloned stocks, we sought to examine whether these viruses represented major species in the viral mix. Genomic DNA was isolated and nested PCR was used to amplify the V1-V2 and V3-V5 regions of HIV-1 envelope gene. These hypervariable regions of *env* gene were selected because most viral heterogeneity has been associated with them. Equal amounts of PCR products were mixed, heat denatured, and then reannealed with analogous fragments derived from standards (SF162 subtype B3 and ZM18 subtype C2) or from JC16 and NC7

molecular clones. Electrophoresis of these fragments on polyacrylamide non-denaturing gels revealed differences in migration rates with homoduplexes moving faster in the gel than heteroduplexes. Homoduplexes based on genomic DNA (HIV-1<sub>JC</sub> or HIV-1<sub>NC</sub>) resulted in homoduplex (lower) and heteroduplex (upper) bands that had comparable intensities and were close together lane. The HIV-1<sub>JC</sub>/HIV-1<sub>JC16</sub> (but not HIV-1<sub>NC</sub>/HIV-1<sub>NC7</sub>) heteroduplex band was between the homoduplex and heteroduplex bands obtained with genomic DNA homoduplexes. The distance between homoduplex and heteroduplex bands formed between JC16 and NC7 molecular clones was larger than that between bands formed from homoduplexes derived from genomic DNA. The SF162 (subtype B3) heteroduplex bands migrated at almost the same rate as the ssDNA but the ZM18 (subtype C2) heteroduplex bands had the slowest migration.

An advantage of establishing a model system for AIDS in smaller nonhuman primates is the decreased cost associated with the housing and upkeep of smaller animals. Animal AIDS models include, but are not limited to, chimpanzees, the gibbons, pig-tailed macaques, and rhesus macaques infected with the HIV-1<sub>JC</sub> or HIV-1<sub>NC</sub> virus of the invention.

Disclosed herein are an immunodeficient pig-tailed macaque and also an immunodeficient rhesus macaque; both infected with the HIV-1<sub>JC</sub> virus of the invention isolated from the C499 chimpanzee which exhibited HIV-1-induced AIDS or the HIV-1<sub>NC</sub> virus isolated from the C455 chimpanzee. The macaques are infected with HIV-1 isolate JC or HIV-1<sub>NC</sub> and are used as an animal model for human AIDS in essentially the same manner as chimpanzees as described above. The transmission of HIV-1<sub>JC</sub> or the HIV-1<sub>NC</sub> virus into a macaque proceeds via injection of an isolated viral suspension or transfection of a biological fluid or tissue specimen from an HIV-1-infected and AIDS-bearing primate. The HIV-1-infected donor specimen is introduced into a recipient by any suitable means, such as intraperitoneal injection, intravenous injection, surgical implantation and combinations thereof. Donor tissue may be introduced as organized tissue (e.g., thymus, lymph node, etc.) or as discrete cells.

Antigenic fragments of the present invention are peptides which contain at least one epitope (antibody binding site) which binds antibodies which bind to at least one HIV-1 isolate of the present invention. The antigenic fragments are preferably capable of inducing an immune response when administered to a nonhuman primate. DNA encoding such antigenic fragments may be used to transform host cells to thereby produce such antigenic fragments.

Antigenic fragments may be identified by a variety of means. A protein from HIV-1<sub>IC</sub> and/or from HIV-1<sub>NC</sub>, such as an envelope protein, may be fragmented with a protease, and the fragments tested to determine whether or not various ones react with antiserum against the protein. See, e.g., J. Robinson et al., *Mol. Cell Biochem.* 21:23-32 (1978). Another technique is to synthesize peptides which are fragments of the entire protein and determine whether the individual fragments are recognized by neutralizing antibodies against the protein. See, e.g., J. Gerin et al., in *Vaccines 85: Molecular and chemical Basis of Resistance to Parasitic, Bacterial and Viral Diseases*, 235-239 (Lerner et al., eds. 1985). Still another method useful for obtaining immunogenic fragments of a protein is by isolation and identification of monoclonal escape mutants. In this strategy, HIV-1 is produced in the presence of a monoclonal antibody to the virus. The only viruses which can grow under these conditions are those with a mutation in the nucleotide sequence which codes for an epitope to which the monoclonal antibody binds. A mutant virus which grows under these conditions is referred to as the "monoclonal escape mutant." The monoclonal escape mutant is then sequenced and the mutant sequence compared with the nucleotide sequence of the HIV-1<sub>IC</sub> isolate or the HIV-1<sub>NC</sub> isolate to find the specific location of the mutation. The mutation is located in a region which codes for a protective epitope, or an "immunogenic fragment." See, e.g., J. Lopez et al., *J. Virol.* 64:927 (1990).

Antigenic preparations of the present invention are useful as reagents in immunoassay diagnostic studies of retroviruses. Immunochemical methods for detecting retroviruses include, for example, immunofluorescence assays or immunoenzymatic assays. Immunofluorescence assays typically involve incubating, for example, serum from the subject to be tested with preparations of the pathogenic virus or fragments thereof. Immune

complexes formed are detected using either direct or indirect methods, for example, the use of antibodies to which fluorescent labels such as rhodamine or fluorescein have been coupled. Immunoenzymatic assays typically involve viral extracts or other antigen-containing compositions bound to a surface. Serum from a subject to be tested for the presence of antibodies directed against one or more antigens is contacted with the surface and, after a period of incubation, unbound substances are washed away. The presence of immune complexes is detected using antibodies labeled with an enzyme such as horseradish peroxidase, alkaline phosphatase, or beta-galactosidase, which is capable of converting a colorless or nearly colorless substrate into a highly colored product, or an enzyme which emits light in the presence of the proper substrate. The amount of product formed is detected visually, spectrophotometrically, or luminometrically and is compared to a similarly treated control. The presence of antibodies in biological fluids may also be detected by agglutination. Viral lysates or antigen compositions are used to coat, for example, latex particles.

Diagnostic tests utilizing the present invention may be carried out in accordance with known techniques. Such techniques provide a method of detecting the presence of HIV-1 by detecting the presence of HIV-1 antibodies. Such methods comprise collecting an antibody-containing biological sample (e.g., blood, blood sera, blood plasma, cerebrospinal fluid, tissue samples) from the subject, contacting the sample with an antigenic preparation of the viral particles of the present invention as given herein, and then detecting the formation of a reaction product between the antibodies in the sample and the antigenic preparation. Any suitable assay format, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) may be employed, in accordance with known techniques. See, e.g., *Immunology: Basic Processes*, 162-175 (J. Bellanti, [Ed.] 2d ed., W.B. Saunders Co. (1985).

Also disclosed herein are kits for the detection of HIV infection. Such kits comprise a container containing an antigenic preparation of the viral particles of the present invention, which may be lyophilized. The antigenic preparation may comprise, e.g., the HIV-1 envelope protein (env) or the group antigen (gag) protein of the HIV-1<sub>IC</sub> and/or HIV-1<sub>NC</sub> of the invention.

A method of inducing antibodies to HIV-1 in a subject, as disclosed herein, comprises administering to a subject an immunogenic amount of infectious viral particles of the present invention. This method may be used to make polyclonal or monoclonal antibodies, which may be used in diagnostic assays. Suitable subjects include mammals (such as, for example, rats, rabbits, mice, and horses) and primates. The term primates is herein intended to encompass any members of the order Primata (for example, lemurs, mandrills, rhesus monkeys, macaques, and chimpanzees) and to include humans. Suitable subjects include those in which antibodies to HIV may be raised (e.g., rabbit, horse).

In the above methods of inducing antibodies, viral antigenic preparations of the present invention may be combined with any suitable pharmaceutically acceptable carrier (such as sterile, pyrogen-free physiological saline solution, or sterile, pyrogen-free phosphate-buffered saline solution). The viral antigens are included in an effective immunogenic amount. The precise amount to be administered to a given subject is determined by techniques known in the art and will vary depending on the route of administration, the subject and the desired response. Administration to the subject may occur by any suitable route (e.g., by intramuscular injection, subcutaneous injection, intraperitoneal injection, or intravenous injection). The appropriate immunogenic dosage will depend upon the particular subject and the desired outcome. Techniques to determine a particular immunogenic amount of the viral particles of the present invention will be apparent to those of ordinary skill in the art. See, e.g., Johnson et al., *Proc. Natl. Acad. Sci. USA* 89:2175 (1992). For example, the active agent (viral particles or preparations thereof) may be given in an amount of from 0.05 to 50  $\mu\text{g}$  per kg body weight (e.g., 0.5 or 1.0  $\mu\text{g}$  per kg).

The invention also provides for a variety of different vaccines based on the structures of the HIV-1<sub>JC</sub> isolate and/or the HIV-1<sub>NC</sub> of the invention and a method for vaccinating a population against HIV. Examples of active agents used for the preparation of a vaccine of the invention include the live attenuated HIV-1<sub>JC</sub> and/or HIV-1<sub>NC</sub> isolate, fixed whole virus, host cells expressing virus antigen, preparations of virus fragments, purified proteins, antigenic fragments of proteins and antigenic peptides which are derivatives of the antigenic fragments. According to the present invention, HIV-1<sub>JC</sub>- and/or HIV-1<sub>NC</sub>-derived

compositions or vaccines are useful for preinfection immunization of primates as well as for postinfection (therapeutic) immunization of HIV-infected primates (see Fultz et al. [1989], Lerner et al., eds. Cold Spring Harbor, NY).

Live attenuated HIV isolate JC virus (or HIV-1<sub>NC</sub> virus) is prepared by serial passage of the virus in tissue culture or genetically altered by recombinant techniques, in accordance with known procedures. Fixed virus is made by contacting live virus (attenuated or unattenuated) to a suitable fixative, such as formalin.

Preparations of viral fragments are made by lysing host cells, such as *E. coli* cells, transformed with a vector encoding an HIV-1 isolate of the present invention (or both) or a portion thereof. The lysate may be used in crude or partially purified form, or a particular viral protein (or antigenic fragment thereof) such as the envelope protein, can be purified to homogeneity and used as an active agent for a vaccine against HIV-1. Host cells such as yeast cells may be transformed with vectors of the present invention capable of expressing HIV-1 proteins, or antigenic fragments thereof, on the surface of the host cells, and the transformed host cells used as an active vaccine agent as such or fixed (e.g., with formalin) and used as an active agent.

Antigenic peptides are selected from the group consisting of antigenic fragments of HIV isolate JC and/or NC proteins, such as the envelope protein, and the antigenic equivalents thereof (i.e., analogs or derivatives). Antigenic peptides may be chemically synthesized or produced by recombinant techniques.

Viral antigenic preparations and cells producing viral antigens and/or fragments thereof may be formulated into immunogenic compositions as neutral or salt forms. Preferably, when cells are used they are of avirulent strains, or the cells are killed before use. Pharmaceutically acceptable salts include but are not limited to the acid addition salts (formed with free amino groups of the peptide) which are formed with inorganic acids, e.g., hydrochloric acid or phosphoric acids; and organic acids, e.g., acetic, oxalic, tartaric, or maleic acid. Salts formed with the free carboxyl groups may also be derived from inorganic

bases, e.g., sodium, potassium, ammonium, calcium, or ferric hydroxides, and organic bases, e.g., isopropylamine, trimethylamine, 2-ethylamino-ethanol, histidine, and procaine.

The term "antigenic equivalents," as used herein, refers to proteins or peptides which bind to an antibody which binds to the protein or peptide with which equivalency is sought to be established. Antibodies which are used to select such antigenic equivalents are referred to as "selection antibodies" herein. Preferred selection antibodies are monoclonal antibodies which bind to HIV isolate JC and/or to HIV-1<sub>NC</sub>, but not to prior isolates of HIV-1 such as the HIV-1 isolates NDK, LAI and SF2, for example.

One or more amino acids of an antigenic peptide sequence may be replaced by one or more other amino acids which do not affect the antigenicity of that sequence. Such changes can be guided by known similarities between amino acids in physical features such as charge density, hydrophobicity/hydrophilicity, size and configuration. For example, threonine and serine can be interchanged, or aspartic acid and glutamic acid, or leucine and isoleucine, and the like.

Antigenic equivalents may be formed by modifying reactive groups within a natural sequence or modifying the N-terminal amino and/or C-terminal carboxyl group. Such equivalents include salts formed with acids and/or bases, particularly physiologically acceptable inorganic and organic acids and bases. Other equivalents include modified carboxyl and/or amino groups on the synthetic peptide to produce esters or amides, or amino acid protecting groups such as N-t-butoxycarbonyl. Preferred modifications are those which provide a more stable, active peptide which will be less prone to enzymatic degradation *in vivo*.

In a particular embodiment of the invention, polyclonal and/or monoclonal antibodies capable of specifically binding to a particular epitope of at least one HIV-1 isolate of the invention are provided. The term *antibody* is used to refer both to a homogenous molecular entity (monoclonal antibody) and a mixture (such as a serum product) made up of a plurality of different molecular entities (polyclonal antibody). Monoclonal or polyclonal antibodies,

and preferably monoclonal, specifically reacting with a particular epitope of interest can be made by methods known in the art. See, e.g., Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories; Goding (1986) *Monoclonal Antibodies: Principles and Practice*, 2d ed., Academic Press, New York. Also, recombinant immunoglobulins may be produced by methods known in the art, including but not limited to the methods described in U.S. Patent No. 4,816,567, incorporated by reference herein. Monoclonal antibodies with affinities of  $10^8 \text{ M}^{-1}$ , preferably  $10^9$  to  $10^{10}$  or more are preferred.

For use as a vaccine, immunogenic compositions may be formulated by any of the means known in the art. Such vaccines are typically prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also, for example, be emulsified, or the protein encapsulated in liposomes. Such vaccines may be administered to the subject by any suitable means, for example, by intramuscular injection, by subcutaneous injection, by intravenous injection, by intraperitoneal injection, by oral injection, and by nasal spray.

The vaccine or other immunogenic composition may be given in a single dose or multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may include 1 to 10 or more separate doses, followed by other doses administered at subsequent time intervals as required to maintain and or reinforce the immune response, e.g., at 1 to 4 months for a second dose and, if needed, a subsequent dose(s) after several months.

The immunogenic peptide antigen compositions are administered in a manner compatible with the dosage formulation and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally in the range of about 100 to 1,000  $\mu\text{g}$  of protein per dose, more generally in the range of about 5 to 500  $\mu\text{g}$  of protein per dose, depends on the subject to be treated, the capacity of the individual's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of the active ingredient required to be administered may depend on the judgment of the physician



and may be peculiar to each individual, but such a determination is within the skill of such a practitioner.

Vaccine formulations of the present invention comprise the active agent mixed with excipients or carriers which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients include but are not limited to water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. The concentration of the immunogenic polypeptide in injectable formulations is usually in the range of 0.2 to 5 mg/ml.

In addition, if desired, the vaccines may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may be effective include but are not limited to: aluminum hydroxide; aluminum phosphate; plant and animal oils; synthetic polymers; e.g., N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP); N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP); N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE); and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate; cell wall skeleton (MPL+TDM+CWS) in a 2% squaline/Tween 80 emulsion; etc.

In addition, the vaccine formulations may also contain one or more stabilizer, for example, carbohydrates such as sorbitol, mannitol, starch, sucrose, dextrin, and glucose, proteins such as albumin or casein, and buffers such as alkaline metal phosphates and the like.

In view of the similarities in protein molecules making up different isolates of HIV-1, the skilled artisan understands that an antibody, particularly a monoclonal antibody, which is specific for a particular epitope directed to a particular protein of the HIV-1<sub>IC</sub> isolate and/or the HIV-1<sub>NC</sub> isolate, can be used to screen for other HIV-1 isolates having similar epitopes recognized by that (monoclonal) antibody.

Antibodies generated against specific epitopes of the HIV-1<sub>JC</sub> of the invention are useful, for example, as probes for screening DNA expression libraries or for detecting the presence of HIV-1 strains in a test sample. Antigens can be synthesized and conjugated to a suitable carrier protein (e.g., bovine serum albumin or keyhole limpet hemocyanin) for use in vaccines or in raising specific antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or noncovalently, a substance which provides a detectable signal. Suitable labels include but are not limited to radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. United States Patents describing the use of such labels include but are not limited to Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,355,241.

Immunogenic carriers may be used to enhance the immunogenicity of an active agent. Such carriers include but are not limited to proteins and polysaccharides, liposomes, and bacterial cells and membranes. Protein carriers may be joined to the capsular polysaccharide molecules to form fusion proteins by recombinant or synthetic means or by chemical coupling. Useful carriers and means of coupling such carriers to polypeptide antigens are known in the art. The art knows how to administer immunogenic compositions so as to generate protective immunity where immunity is most helpful.

Compositions and immunogenic preparations including vaccine compositions comprising substantially purified antigens derived from an HIV-1 isolate JC and/or NC and a suitable carrier therefor are provided. Immunogenic compositions are those which result in specific antibody production when injected into a human or an animal. Such immunogenic compositions are useful, for example, in immunizing primates against infection by HIV-1 strains. The immunogenic preparations comprise an immunogenic amount of, as specifically exemplified, at least one antigenic determinant derived from the HIV-1<sub>JC</sub> isolate of the invention and a suitable carrier. Alternatively, the immunogenic composition can comprise host cells harboring an antigenic agent from the specifically exemplified HIV-1<sub>JC</sub> strain and a suitable carrier. It is understood by one of ordinary skill in the art that a functionally equivalent, recombinant mutant of HIV-1<sub>JC</sub> and or HIV-1<sub>NC</sub> can be produced by the

introduction of the cloned DNA containing the insertion mutations responsible for a desired characteristic. It is also within the scope of the present invention and readily within the grasp of the ordinary skilled artisan to generate other types of genetically stable mutations in the structural or enzyme genes of HIV-1. Such immunogenic compositions (or vaccines) are useful, for example, in immunizing an animal, especially humans, against AIDS and related diseases resulting from infection by HIV-1 species. Such immunogenic compositions can also elicit the production of antibodies which will cross react with proteins of other HIV-1 and HIV-2 strains expressing epitopes in common with those of the starting HIV-1<sub>JC</sub> isolate. It is understood that where whole cells are formulated into the immunogenic composition, the cells are preferably inactivated, especially if the cells are of a virulent strain. Such immunogenic compositions may comprise one or more protein or the immunogenic cellular component. By "immunogenic amount" is meant an amount capable of eliciting the production of antibodies directed against an antigenic agent of HIV-1<sub>JC</sub> in an animal or human to which the vaccine or immunogenic composition has been administered.

The nucleotide sequence of the HIV-1<sub>JC</sub> isolate or the HIV-1<sub>NC</sub> isolate can be used to generate hybridization probes which specifically bind to HIV-1<sub>JC</sub> genetic material, or to DNA of HIV-1 isolates having the identifying characteristics of the HIV-1 isolates JC or NC, to determine the presence of such HIV-1 in primates. The hybridization probe may be selected so that it does not bind to other known HIV-1 isolates such as NDK, LAI, SF2, etc. The hybridization probes can be cDNA fragments or polynucleotides and may be labeled with a detectable group, as is well-known in the art. Pairs of probes can serve as PCR primers for synthesis and amplification processes in accordance with the description, for example in U.S. Patent Nos. 4,683,202 and 4,683,195.

In specific embodiments, probes of the invention comprise DNA sequences of HIV-1<sub>JC</sub> or sequences encoding antigenic fragments thereof or sequences having identity thereto. In particular are provided probes having a DNA sequence as set forth in SEQ ID NO:1 or a sequence having identity thereto.

The production of DNA, vectors, transformed host cells, HIV-1 virus, proteins, and protein fragments of the present invention by genetic engineering techniques can be carried out in accordance with methods known in the art. See, e.g., U.S. Pat. No. 4,761,371, U.S. Pat. No. 4,877,729, U.S. Pat. No. 4,912,038, and U.S. Pat. No. 4,879,224, among others.

A nucleotide sequence (polynucleotide) or fragment thereof is substantially homologous (or substantially similar) to another polynucleotide if, when optimally aligned (with appropriate nucleotide insertions or deletions) with another polynucleotide, there is nucleotide sequence identity for approximately 80% of the nucleotide bases, usually approximately 90%, more preferably about 95% to 100% of the nucleotide bases. Gaps introduced to optimize alignments are treated as mismatches.

Alternatively, substantial homology (or similarity) exists when a polynucleotide or fragment thereof will hybridize to another polynucleotide under selective or stringent hybridization conditions. Selectivity of hybridization exists under stringent hybridization conditions which allow one to distinguish the target polynucleotide of interest from other polynucleotides. Typically, selective hybridization will occur when there is approximately 75% similarity over a stretch of about 14 nucleotides, preferably approximately 80% similarity, more preferably approximately 85% similarity, and most preferably approximately 90% similarity. See Kanehisa (1984) *Nucl. Acids Res.*, 12:203-213. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will often be over a stretch of about 17 to 20 nucleotides, preferably 21 to 25 nucleotides, more preferably 26 to 35 nucleotides, and more preferably about 36 or more nucleotides.

The hybridization of polynucleotides is affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing polynucleotides, as will be readily appreciated by those skilled in the art. Stringent temperature conditions will generally include temperatures in excess of 30°C, typically in excess of 37°C, and preferably in excess of 45°C. Stringent salt conditions will ordinarily be less than 1 M, typically less than 500 mM, and preferably less than 200 mM.

However, the combination of parameters is much more important than the measure of any single parameter (Wetmur and Davidson [1968] *J. Mol. Biol.* 31:349-370).

An isolated or substantially pure polynucleotide is a polynucleotide which is substantially separated from other polynucleotide sequences which naturally accompany a native sequence. The term embraces a polynucleotide sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates, chemically synthesized analogues and analogues biologically synthesized by heterologous systems.

A polynucleotide is said to encode a polypeptide if, in its native state or when manipulated by methods known to those skilled in the art, it can be transcribed and/or translated to produce the polypeptide of a fragment thereof. The anti-sense strand of such a polynucleotide is also said to encode the sequence.

Vectors are replicable DNA constructs used to either amplify or express DNA of the present invention. An expression vector is a replicable DNA construct in which DNA of the present invention is operably linked to control sequences capable of expressing that DNA in a suitable host. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation. Suitable vectors include plasmids, viruses (e.g., vaccinia virus, adenovirus, baculovirus, cytomegalovirus) phage, and integratable DNA fragments (i.e., fragments integratable into the host genome by recombination).

DNA regions are operably linked or operably associated when they are functionally related to each other. For example, a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation.

Transformed host cells are cells which have been transformed or transfected with vectors as described above. Transformed host cells ordinarily express the DNA of the present invention. Suitable host cells include prokaryote, yeast or higher eukaryotic cells such as mammalian cells and insect cells.

Prokaryote host cells include gram negative or gram positive organisms, for example *Escherichia coli* (*E. coli*) or Bacilli. Exemplary host cells are *E. coli* W3110 (ATCC 27,325), *E. coli* B, *E. coli* X1776 (ATCC 31,537), *E. coli* 294 (ATCC 31,446). A broad variety of suitable prokaryotic and microbial vectors are available. *E. coli* is typically transformed using pBR322. Promoters most commonly used in recombinant microbial expression vectors include the  $\beta$ -lactamase (penicillinase) and lactose promoter systems (Change et al., *Nature* 275:615 [1978]; and Goeddel et al., *Nature* 281:544 [1979], a tryptophan (*trp*) promoter system (Goeddel et al. [1980] *Nuc. Acids Res.* 8:4057, and EPO App. Publ. No. 36,766) and the *tac* promoter (H. De Boer et al., *Proc. Natl. Acad. Sci. USA* 80:21 [1983]). The promoter and Shine-Dalgarno sequence are operably linked to the DNA of the invention, i.e., they are positioned so as to promote transcription of messenger RNA from the DNA.

Eukaryotic microbes such as yeast cultures may also be transformed with vectors of the present invention. See, e.g., U.S. Pat. No. 4,745,057. *Saccharomyces cerevisiae* is the most commonly used yeast, although other yeast may also be used. Host cells such as insect cells (e.g., cultured *Spodoptera frugiperda* cells) and expression vectors such as the baculovirus expression vector may be employed in carrying out the present invention, as described in U.S. Pat. Nos. 4,745,051 and 4,879,236 to Smith et al.

Examples of useful mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and WI138, BHK, COS-7, CV, and MDCK cell lines. The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells are often provided by viral sources. For example, commonly used promoters are derived from polyoma, Adenovirus 2, and Simian Virus 40 (SV40). See, e.g., U.S. Pat. No. 4,599,308. An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral

(e.g., Polyoma, Adenovirus, VSV, or BPV) source, or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter is often sufficient. Rather than using vectors which contain viral origins of replication, one can transform mammalian cells by the method of cotransformation with a selectable marker and DNA of the present invention, as described in U.S. Pat. No. 4,399,216.

Except as noted hereafter, standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al. (1989) *Molecular Cloning*, Second Edition, Cold Spring Harbor Laboratory, Plainview New York; Maniatis et al. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) *Meth. Enzymol.* 218:Part I; Wu (ed.) (1979) *Meth. Enzymol.* 68; Wu et al. (eds.) (1983) *Meth. Enzymol.* 100 and 101; Grossman and Moldave (eds.) *Meth. Enzymol.* 65; Miller (ed.) (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Plainview, New York; Old and Primrose (1981) *Principles of Gene Manipulation*, University of California Press, Berkely; Schleif and Wensink (1982) *Practical Methods in Molecular Biology*; Glover (ed.) (1985) *DNA Cloning* Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) *Nucleic Acid Hybridization*, IRL Press, Oxford, UK; and Setlow and Hollaender (1979) *Genetic Engineering: Principles and Methods*, Vols. 1-4, Plenum Press, New York. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

All publications, patent applications and patents cited herein are incorporated by reference to the extent that they are not inconsistent with the present disclosure.

The foregoing discussion and the following examples are provided for illustrative purposes, and they are not intended to limit the scope of the invention as claimed herein. Modifications and variations which may occur to one of ordinary skill in the art are within the

intended scope of this invention. The present invention is further described in the non-limiting examples set forth below.

## EXAMPLES

### Example 1. Animal Subjects

All animals (e.g., chimpanzees) were maintained in accordance with the guidelines established by the Animal Welfare Act and the NIH guide for care and use of laboratory animals. The Yerkes Center is fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC).

A cohort of 12 chimpanzees was inoculated with several strains of HIV-1 at the Yerkes Center. A member of this cohort, chimpanzee 499 was inoculated on three different occasions with three different HIV-1 isolates: HIV-1<sub>SF2</sub> in 1985, HIV-1<sub>LAI</sub> in 1986 and HIV-1<sub>NDK</sub> in 1987 (See Fultz et al. [1991] J. Infect. Dis. 163:441-447 and Novembre et al. [1997] J. Virol. 71:4086-4091).

It is preferred that a chimpanzee be bred in captivity, be seropositive for Epstein-Barr virus and cytomegalovirus and be seronegative and PCR negative for HIV prior to being used as an animal model.

### Example 2. Isolation and Purification of HIV-1<sub>JC</sub> Isolate

The original source of the HIV-1<sub>JC</sub> isolate was the chimpanzee C499. HIV was easily isolated from the peripheral blood mononuclear cells (PBMC) of this animal. Cocultivation of PBMC derived from C499 with uninfected chimpanzee PBMC (cPBMC) resulted in the isolation of a virus (HIV-1<sub>JC</sub>) which induced syncytium formation in chimpanzee cells. The nucleotide sequence of this virus was distinguished from the isolates used for the initial inoculations and from other known HIV-1 isolates.

The HIV-1<sub>JC</sub> isolate was deposited with the AIDS Reagent Program, McKesson Bioservices, 685 Lofstrand Lane, Rockville, MD 20850 USA, a division of the NIH AIDS



Research and Reference Reagent Program, in May 1997 and has been assigned Catalog Number 3523. A substantially full-length infectious molecular clone has a nucleotide sequence as given in SEQ ID NO:11.

In a specific embodiment of the invention, probes of the invention comprise DNA sequences as set forth in SEQ ID NO:11 and/or sequences of at least 15 contiguous nucleotides derived therefrom or sequences complementary thereto.

The original source of the HIV-1<sub>JC</sub> isolate was the chimpanzee C455. HIV was easily isolated from the peripheral blood mononuclear cells (PBMC) of this animal. Cocultivation of PBMC derived from C455 with uninfected chimpanzee PBMC (cPBMC) resulted in the isolation of a virus (HIV-1<sub>NC</sub>) which induced syncytium formation in chimpanzee cells. The nucleotide sequence of this virus is distinguished from other known HIV-1 isolates. A substantially full-length infectious molecular clone has a nucleotide sequence as given in SEQ ID NO:12.

In a specific embodiment of the invention, probes of the invention comprise DNA sequences as set forth in SEQ ID NO:12 and/or sequences, of at least 15 contiguous nucleotides derived therefrom or sequences complementary thereto.

### Example 3. Virus and Viral Antigens

The HIV-1 isolates used to inoculate C499 included LAV (lymphadenopathy-associated virus)-1<sub>BRU</sub> (Barre-Sinoussi [1983] Science 220:868-871), SF2 (previously designated ARV [AIDS-related virus]-2) (Levy et al. [1984] Science 225:840-842), and NDK, a highly cytopathic HIV-1 of African origin (Spire et al. [1989] Gene 81:275-284). Inoculations of virus were done intravenously with 1-ml aliquots of undiluted or diluted virus stocks. In January 1988, 33 months after inoculation HIV-1<sub>SF2</sub>, C499 was part of a study to assess the effects of therapeutic vaccination on immunity and viral status in HIV-1-infected chimpanzees. C499 was given two intramuscular injections, 4 weeks apart, of 500 µg of recombinant SF2 p53 gag, produced in yeast and formulated with 100 µg of muramyl

tripeptide (Ciba-Geigy, Summit, NJ) in 4% squalene and 0.008% Tween 80 (Fultz et al. [1989] in *Vaccines* 89, *supra*).

The HIV-1 isolates used to inoculate C455 was JC, deposited with the AIDS Reagent Program, Catalog Number 3523. Inoculations of virus were done intravenously with 1-ml aliquots of undiluted or diluted virus stock. C534 was given two intramuscular injections, 4 weeks apart, of 500 µg of recombinant SF2 p53 *gag*, produced in yeast and formulated with 100 µg of muramyl tripeptide (Ciba-Geigy, Summit, NJ) in 4% squalene and 0.008% Tween 80 (Fultz et al. [1989] in *Vaccines* 89, *supra*).

#### Example 4. Serologic Assays

Serum samples were tested by indirect ELISA for antibodies to specific HIV-1 proteins using a series of recombinant antigens. These antigens have been described (Fultz et al. [1989] in *Vaccines* 89, *supra*) and included p25 *gag* and p53 *gag*, produced in yeast; p31 *pol*; and *env*2-3(SF2) and *env*2-3 (IIIB), nonglycosylated polypeptides corresponding to full-length gp120. Antibody titers to whole-virus preparations were determined with an HIV enzyme immunoassay kit (EIA; Genetic Systems, Seattle, WA). Neutralization assays were performed as described (Fultz et al. [1986] *Proc. Natl. Acad. Sci.* 83:5286-5290); titers were based on >80% inhibition of reverse transcriptase (RT) activity after preincubation of virus with serum and infection of normal human PBMC. Antibodies cross-reactive with histone H2B were identified by immunoblot using purified calf thymus histones as described previously (Strickler et al. [1987] *Nature* 327:710-713).

#### Example 5. Virus Assays

Assays to detect virus in plasma samples or to recover virus from chimpanzee PBMC were performed with normal human PBMC as indicator cells, unless otherwise indicated, and have been described (Fultz et al. [1986] *J. Virol.* 58:116-124). Medium was RPMI 1640 with 10% fetal bovine serum, 8 units of recombinant interleukin-2 (IL-2)/ml, glutamine, and antibiotics (RPMI-IL-2). Infectious PBMC were quantified by serial 1:5 limiting dilution and cocultivation with human PBMC (Fultz et al. [1986], *supra*). HIV-1 was detected in all assays by the presence of RT activity in cell-free culture supernatants. To detect HIV antigen in

serum or plasma samples, a commercially available HIV-1 antigen capture kit (Coulter, Hialeah, FL) was used.

To assess the influence of CD8<sup>+</sup> lymphocytes on viral replication and recovery, after 3 days of stimulation with concanavalin A (ConA 10 µg/ml) in RPMI 1640, PBMC were washed once; 10<sup>7</sup> cells from C499 were placed in fresh RPMI-IL-2, cultured without indicator cells, and monitored for HIV-1 production. In other experiments, the overnight incubation of C499's PBMC to remove adherent cells, CD4<sup>+</sup>- and CD8<sup>+</sup>-enriched populations were obtained by panning with monoclonal antibodies specific for the CD8<sup>+</sup> antigen or with CD8<sup>+</sup>-coated magnetic beads (Dynabeads; Robbins Scientific, Mountain View, CA). After removal of the CD8<sup>+</sup> cells, CD8<sup>+</sup> lymphocytes were recovered from the plates or magnetic beads by additional overnight incubation and repeated washes with medium. After stimulation with ConA, cultures were established either with CD4<sup>+</sup>-enriched cells only or with CD4<sup>+</sup>- and CD8<sup>+</sup>-enriched cells in various ratios. Percentages of CD4<sup>+</sup> and CD8<sup>+</sup> cells in enriched populations were determined by analysis with FACScan (Becton-Dickinson, Mountain View, CA).

Replication kinetics in PBMC and macrophages were tested as follows. 1 x 10<sup>7</sup> freshly isolated or Con-A stimulated PBMC from HIV-1 negative chimpanzee were infected overnight (at 37°C) with 20 ng of the indicated virus (p24 antigen concentration). The cells were centrifuged at 1000 rpm for 10 min, resuspended in 10 ml complete RPMI medium containing 10% FBS and IL-2 (IL-2 medium) and were incubated at 37°C. Samples of supernatants (1 ml) were harvested on days 3, 7, 10, 14 and 17 post infection. IL-2 medium was added to the cultures following the sampling to maintain the original volume. Supernatants were used in RT assays to determine the relative amounts of virus produced.

For replication in macrophages, cPBMC were resuspended in macrophage media (6 x 10<sup>6</sup>/well in RPMI 1640 containing 15% human serum [AB<sup>+</sup>], 1% HEPES, 0.008 ng/ml GM-CSF, 0.03 ng/ml M-CSF, 1% antibiotic-antimycotic solution (Sigma, St. Louis, MO) and seeded in a 24-well plate and incubated at 37°C for 4 hrs. The cells were mixed by pipetting up and down before the incubation was continued for 4 days. Non-adherent cells were

removed by gently washing the wells. Fresh medium (2 ml/well) was added and the cells were cultured for an additional 3 days to allow full macrophage differentiation. Infections were initiated by adding 10 ng of virus (p24) to the cells in 500  $\mu$ l media and adsorbed overnight. The inoculum was removed and the cells were washed twice before fresh macrophage medium (2 ml) was added. On days 7 and 14 post infection, aliquots of 0.5 ml were taken for determination of p24 antigen levels using the HIV-1 p24 Antigen kit (Coulter Corp, Miami, FL) according to the manufacturer's instructions.

**Example 6. Cell-mediated Immune Responses**

Lymphocyte proliferative responses to mitogens were tested by incorporation of [ $^3$ H]thymidine into the DNA of PBMC seeded in triplicate at  $5 \times 10^4$  PBMC per well into 96-well plates. PBMC were incubated for four days with different concentrations of phytohemagglutinin (PHA) or ConA, pulsed overnight with 1  $\mu$ Ci of [ $^3$ H]thymidine per well, and harvested with a cell harvester (Skatron, Sterling, VA); counts per minute (cpm) incorporated were determined in a  $\beta$  counter.

Cytotoxic T lymphocyte (CTL) responses against HIV-1 *env*- and *gag*-encoded antigens were assayed using fresh PBMC from C499 as effector cells and autologous Epstein Barr virus-transformed B cells as target cells. Target cells were infected at MOI=10 with recombinant vaccinia viruses expressing HIV-1 gpl 20 *env* of p24 *gag* or with wild-type vaccinia as control. Infected targets were labeled with 200  $\mu$ Ci of [ $^{51}$ Cr] sodium chromate (Du Pont, Boston) for 2 hours and washed, and  $10^5$  target cells (T) were mixed with effector cells (E) at various ratios in 96-well plates. The plates were incubated for 6 hours at 37°C in 5% CO<sub>2</sub>, then 0.1 ml of medium was removed from each well and counted in a  $\gamma$  counter. Maximum release (max) was determined by adding 1% Triton X to labeled target cells, and spontaneous release (spon), by adding only medium. Percentage of specific lysis was determined:  $[\text{cpm}(\text{experimental}) - \text{cpm}(\text{spon})] / [\text{cpm}(\text{max}) - \text{cpm}(\text{spon})] \times 100$ .

**Example 7. Nonhuman Primate Model Systems for AIDS**

Nonhuman primates infected with HIV-1 isolate JC or infected with HIV-1<sub>NC</sub> are useful as a model system for the study of AIDS. Chimpanzees and other monkey species

used for this purpose are preferably specific pathogen-free animals, which are available from primate centers, e.g., the Yerkes Regional Primate Research Center, Emory University, Atlanta, GA.

Infected primates are preferably maintained as a single colony of two or more animals, all inoculated with HIV-1 isolate JC or a colony of two or more animals, all inoculated with HIV-1<sub>NC</sub>. A colony may be maintained in a single room with each primate housed in an appropriate cage, in accordance with standard practices for the maintenance of animals established by the Animal Welfare Act and the NIH guide for care and use of laboratory animals.

The primates are infected with the HIV-1<sub>JC</sub> virus or the HIV-1<sub>NC</sub> virus by any suitable means, such as intraperitoneal, intravenous or subcutaneous injection with a solution containing HIV-1 isolate JC or HIV-1<sub>NC</sub>. The solution may also be a body fluid or tissue (e.g., blood) from a previously infected primate, a blood fraction containing peripheral blood mononuclear cells from a previously infected primate, a pharmaceutically acceptable carrier such as saline solution containing HIV-1 isolate JC or HIV-1 isolate NC, etc.

Nonhuman primates infected with HIV-1<sub>JC</sub> or HIV-1<sub>NC</sub> are particularly useful as a model system for AIDS because of the concomitant decrease in CD4<sup>+</sup> cells and increase in HIV-1 loads in plasma. The development of AIDS has not been previously documented in any nonhuman primate species. When used as a model system, a primate(s) infected with HIV-1<sub>JC</sub> or HIV-1<sub>NC</sub> virus is subjected to a treatment useful in combating AIDS in humans and thereafter the progress of the infection and related diseases is monitored. A control (placebo) group of HIV-1<sub>JC</sub> or HIV-1<sub>NC</sub>-infected animals is left untreated for comparative purposes. A slowing in the progression of the development of AIDS in infected animals indicates that the treatment may be useful for combating AIDS in humans and additional screening and toxicological testing is prescribed. Such treatment includes but is not limited to a vaccine, a drug (e.g., an antiretroviral compound) or a drug combination (e.g., antiviral nucleosides such as AZT, DDI, etc.), a peptide, a protein, etc.) or a vaccine/drug combination, etc. After treatment initiation, the progress of the disease is monitored by any suitable

parameters including, but not limited to, (a) decline in CD4<sup>+</sup> cell levels, (b) increase in viral loads in plasma, (c) presence of HIV-1<sub>JC</sub> virus or HIV-1<sub>NC</sub> virus, (d) weight loss, (e) general appearance, and other symptoms characteristic of AIDS.

#### Example 8. Virus Cloning and Sequence Analysis

cPBMIC infected with either HIV-1<sub>JC</sub> or HIV-1<sub>NC</sub> were used for isolation of DNA using the Puregene Kit (Gentra systems, Minneapolis, MN) as directed by the manufacturer. The strategy used for PCR amplification and primer location on the HIV-1 viral genome is shown in Figure 3. PCR primers MSF12 (5'-AAA TCT CTA GCA GTG GCG CCC GAA CAG-3', (SEQ ID NO:18); HIV-1<sub>LAV</sub> nt 169 to 195) and MSR5 (5'-GCA CTC AAG GCA AGC TTT ATT GAG GCT-3', (SEQ ID NO:19); HIV-1<sub>LAV</sub> nt 9225 to 9198) [Salminen et al. (1995) *Virology* 213, 80-86] were used to amplify a 9056 bp product from PBMIC genomic DNA prepared from HIV-1<sub>JC</sub>-infected cells. Another PCR primer pair 527 (5'-CAC ACA CAA GGC TAC TTC CCT GAT TGG CAG A-3', SEQ ID NO:20, HIV-1<sub>LAV</sub> nt 5302 to 5274) was used to amplify 5' LTR-containing fragments (5699 bp) from the same DNA source. For the HIV-1<sub>NC</sub> viral genome, PCR primer pairs 527-528 and 529 (5'-ATG GAA CAA GCC CCA GAA GAC CAA GGG CCA CAG-3', SEQ ID NO:21, HIV-1<sub>LAV</sub> nt 5141 to 5173) and 530 (5'-GGT CTG AGG GAT CTC TAG TTA CCA GAG TCA C-3', SEQ ID NO:22, HIV-1<sub>LAV</sub> nt 151 to 121) were used to generate the 5'-half (5699 bp) and 3'-half (4142 bp) PCR products respectively from HIV-1<sub>NC</sub> genomic PBMIC DNA. Primers were synthesized on an Applied Biosystems 392 DNA synthesizer (Applied Biosystems, Foster City, CA). Briefly, PCR was performed using the reagents from the Expand Long Template kit (Boehringer Mannheim, Indianapolis, IN) and 200 ng of DNA template, according to the manufacturer's instructions. After an initial DNA denaturation of 94°C for 2 min, the PCR consisted of 10 cycles of 94°C for 15s, 61°C for 30s, 68°C for 8 min followed by 20 cycles of 94°C for 15s, 61°C for 30s, and 68°C for 8 min with a 5 second addition to each extension. The samples were incubated at 72°C for 30 min after the last cycle and then cooled to 4°C. Results of PCR reactions were evaluated on 0.9% agarose gels. PCR products representing the correct sized fragments were isolated from agarose and were directly cloned into the pCR II plasmid and amplified in *Escherichia coli* bacteria (TA cloning kit, Invitrogen Corp., San Diego, CA) according to the manufacturer's protocol.

Single bacterial colonies containing plasmids with inserts of the correct size were grown at 30°C overnight and plasmid DNA prepared by the alkaline lysis method.

The strategy for preparing full-length molecular clones is illustrated in Figure 3. Several restriction enzymes were used to generate restriction maps for the positive clones. The *Apa* I fragment (1947 bp) from the 5' half PCR product was gel-purified and subcloned into the large fragment (7675 bp) of pJC using standard cloning procedures [Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY] to generate plasmid pHIV-1<sub>JC16</sub> (Fig. 1). A chimeric plasmid (pHIV-1<sub>NCJC</sub>) was generated by subcloning the PCR amplified 5' half of HIV-1<sub>NC</sub> into pHIV-1<sub>JC16</sub>. For HIV-1<sub>NC</sub> full-length clone, the *Nco* I-*Xho* I *env*-containing fragment from the 3' half PCR product was gel purified and subcloned into the *Nco* I-*Xho* I large fragment of plasmid pHIV-1<sub>NCJC</sub> containing the 5' half of HIV-1<sub>NC</sub>. Multiple restriction enzymes were used for analysis of both viral DNAs to confirm the full length clones.

5 x 10<sup>6</sup> CEMx174 cells in T-25 flasks were transfected with 2 µg of either pHIV-1<sub>JC16</sub> or NC7 DNA in transfection buffer (25 mM Tris-HCl)(pH 7.5, 140 mM NaCl, 5 mM KCl, 0.7 mM K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O) containing 4 µl of DEAE-dextran (60 mg/ml) for 20 minutes at room temperature. Five milliliters of complete medium (RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine) was added to stop the reaction followed by centrifugation of cells at 1000 rpm for 10 minutes. The cells were washed twice in 10 ml complete medium before they were resuspended in 10 ml complete medium, transferred to a T-25 cm<sup>2</sup> flask and incubated at 37°C (5% CO<sub>2</sub>). The cells were checked daily for cytopathic effects (syncytia formation) and aliquots of cultures were tested for the presence of reverse transcriptase (RT) activity using standard assay methods. For 293 cell lines, 2 x 10<sup>5</sup> cells in 6-well plates were transfected with 2 µg of viral DNA using lipofectin (Life Technologies, Gaithersburg, MD) or DOTAP (Boehringer Mannheim, Indianapolis, IN) according to the manufacturers' instructions. After 24 hours, the transfected cells were overlaid with 2 x 10<sup>6</sup>/well of uninfected cPBMC previously stimulated with concanavalin A (Con-A) for 4 days. After an additional 2 day incubation, the non-adherent cell population (cPBMC) were transferred to a T-25 flask and additional stimulated cPBMC added for virus amplification.

Culture supernatants were assayed for RT activity and the cells were observed daily for development of syncytia. Cell-free stocks of molecularly cloned viruses were prepared at peak RT activity, aliquoted and stored under liquid nitrogen.

Primers for sequencing were constructed from conserved regions of aligned sequences of HIV-1<sub>LAI</sub> and HIV-1<sub>SF2</sub> and were synthesized on an applied Biosystems 392 DNA synthesizer. The DNA sequence of each full-length clone virus was determined by the dideoxy-chain termination method using the sequenase system (Amersham Life Sciences, Arlington Heights, IL) and <sup>35</sup>S-dATP. Nucleotide sequence alignments were performed with the Intelligenetics Suite of programs (Intelligenetics, Beaverton, OR) while the phylogenetic analysis of amino acid sequence was done with CLUSTAL method (DNASTAR, Inc., Madison, WI).

#### Example 9. Heteroduplex Mobility Assays.

The nucleotide sequences for HIV-1<sub>IC16</sub> and HIV-1<sub>NC7</sub> have been assigned Genbank accession numbers AF049494 and AF049495 respectively.

The heteroduplex mobility assay kit (NIH AIDS Research and Reference Reagent Program) based on the method described by Delwart et al. (1993) *Science* 262, 1257-1261 was used. Briefly, equal amounts (5 µl each) of second-round PCR products (V1-V2 and V3-V5) from infected cPBMC genomic DNA were mixed with the reference PCR products to obtain heteroduplexes. After adding 1.1 µl of 10x annealing buffer (1 M NaCl, 100 mM Tris [pH 7.8], 20 mM EDTA), the mixed DNAs were denatured at 94°C for 2 min and then reannealed by rapidly cooling in ice. Three µl of loading dye (25% Ficoll, 1% Orange G) was added to the cooled DNA mixture and the samples loaded onto 5% polyacrylamide gel in 1x TBE (88 mM Tris-borate, 89 mM boric acid, 2 mM EDTA) buffer and electrophoresed at a constant voltage of 250 V for 2.5 hrs. The gels were stained with ethidium bromide and visualized under ultra-violet (UV) light.



TABLE 1

Platelet counts, lymphocyte subset levels, and plasma HIV-1 loads in C499

Date (mo/day/yr)	Platelet count (cells/ $\mu$ l)	Total lymphocyte count (cells/ $\mu$ l) <sup>a</sup>	Absolute CD4 <sup>+</sup> cell count (cells/ $\mu$ l)	Absolute CD8 <sup>+</sup> cell count (cells/ $\mu$ l)	Plasma virus level (RNA equivalents/ml) <sup>b</sup>
10/04/88	63,000	3,136	550	1,020	<10 <sup>4</sup>
06/12/89	122,000	3,360	810	1,750	<10 <sup>4</sup>
01/02/90	112,000	3,600	1,040	1,690	<10 <sup>4</sup>
02/06/90	289,000	5,301	1,480	2,490	<10 <sup>4</sup>
05/11/93	24,000	2,461	390	1,720	0.86 X 10 <sup>5</sup>
05/18/94	23,000	1,430	300	770	ND <sup>c</sup>
09/11/95	23,000	2,312	160	1,500	1.27 x 10 <sup>5</sup>
09/13/95	12,000	532	10	230	1.84 x 10 <sup>5</sup>
09/27/95	20,000	4,508	180	4,060	1.65 x 10 <sup>5</sup>
10/19/95	12,000	3,854	120	2,960	1.21 x 10 <sup>5</sup>
11/16/95	5,000	5,616	170	4,660	1.05 x 10 <sup>5</sup>
12/19/95	20,000	1,920	60	1,520	1.19 x 10 <sup>5</sup>
01/23/96	12,000	4,366	90	3,150	1.07 x 10 <sup>5</sup>
02/13/96	12,000	11,232	110	6,510	0.90 x 10 <sup>5</sup>

<sup>a</sup> Lymphocyte subset counts in peripheral blood were determined by FACScan analysis as described previously (Ahmed-Ansari et al. [1989] Am. J. Primatol. 17:107-131).

<sup>b</sup> Virus levels were determined using the Chiron B-DNA assay as directed by the manufacturer. Plasma samples were stored at - 80°C until use.

<sup>c</sup> ND, not done (no plasma sample was available for this date).

[illegible]

[illegible]

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Table 2B. Alignment of HIV-1<sub>JC</sub> and HIV-1<sub>LAJ</sub> *env* Protein Sequences (SEQ ID NO:2 and SEQ ID NO:4, respectively). The consensus sequence corresponds to SEQ ID NO:7.

HIVJCENV	1	MRVKEncQHLWRWGKWKgIMLLGmLMICSATEKLWVTVYYGVPVWKEtTTTLFCASDAKAY
HIVlaienvt	1	MRVKEkyQHLWRWGKWKgIMLLGmLMICSATEKLWVTVYYGVPVWKEaTTTLFCASDAKAY
consensus		MRVKE--QHLWRWGKWKg-MLLG-LMICSATEKLWVTVYYGVPVWKE-TTTTLFCASDAKAY
HIVJCENV	62	eeEVHNVWATHACVPTDPNPQeIVLanVTedFNMWKNemVEQMHeDIISLWDeSLKPCVKL
HIVlaienvt	62	dtEVHNVWATHACVPTDPNPQeVLvNVTEnFNMWKNdMVEQMHeDIISLWDqSLKPCVKL
consensus		--EVHNVWATHACVPTDPNPQe-VL-NVTE-FNMWKN-MVEQMHe-DIISLWD-SLKPCVKL
HIVJCENV	123	TPLCVtLnCTDLkNeTkTNSSdaNSnSGEiM gneEIKNCSFNvSTgapGKVQKEYaIFYa
HIVlaienvt	123	TPLCVsLkCTDLgNaTnTNSSntNSsSGEmMmekgEIKNCSFNiSTsirGKVQKEYafFYk
consensus		TPLCV-L-CTDL-N-T-TNSS--NS-SGE-Mm---EIKNCSFN-ST---GKVQKEY--FY-
HIVJCENV	183	LDIvsIkneNnsTShmLTSCNTSVstQACPKVSFEPIPIHYCAPAGFAILKCNdKkFNGTG
HIVlaienvt	184	LDI IpidNdtTSytLTSCNTSVitQACPKVSFEPIPIHYCAPAGFAILKCNnKtFNGTG
consensus		LDIvsI---N--TS--LTSCNTSV-TQACPKVSFEPIPIHYCAPAGFAILKCN-K-FNGTG
HIVJCENV	244	PCnNVSTVQCTHGIRPVVSTQLLLLNGSvAEEEVVlRSANFsdNAKTIIVQLNhsVEItCTR
HIVlaienvt	243	PcNnVSTVQCTHGIRPVVSTQLLLLNGSLAEEEVVlRSANFtdNAKTIIVQLNqSVEInCTR
consensus		PC-NVSTVQCTHGIRPVVSTQLLLLNGS-AEEEVV-RSANF-DNAKTIIVQLN-SVEI-CTR
HIVJCENV	305	PNyNetKkIRIhRGyGRsFVTvrKlGdrkQAHCTmmRtKWdnaLKQIASKLREQFgNktaI
HIVlaienvt	304	PNNtrKsIRIqRGpGRaFVTigKiGnmrQAHcNisRaKWnatLKQIASKLREQFgNnktI
consensus		PN-N--K-IRI-RG-GR-FVT--K-G---QAHc---R-KW---LKQIASKLREQFgN---I
HIVJCENV	365	IFnrSSGGDIEiemHSFNCGGEIfYCNtTkLFNSTW NeT TE SN gkG enITLPCRI
HIVlaienvt	365	IFkqSSGGDpEIvtHSFNCGGEIfYCNsTqLFNSTWfNsTwsTEgSNnteGsdITLPCRI
consensus		IF--SSGGD-EI--HSFNCGGE-FYCN-T-LFNSTWfN-TwsTEgSNn--Gs--ITLPCRI
HIVJCENV	420	rQFvNMWQkvVGKAMYAPPsdGQIRcTSNITGLLLTRDGGHndNNtnnEtFRPGrGDMDRDNW
HIVlaienvt	426	kQFiNMWQeVGKAMYAPPisGQIRCSNITGLLLTRDGG N NNngsEiFRPGgGDMDRDNW
consensus		-QF-NMWQ-VGKAMYAPP--GQIRC-SNITGLLLTRDGGHndNN---E-FRPG-GDMDRDNW

Table 2B. (Continued)

HIVJCENV	481	RSELYKYKV	KIEPLGVAPT	KAKRRVVQREKRAVG	Gm	GAm	FLGFLGAAGSTMGA	S	TLTV
HIVlaienvt	485	RSELYKYKV	KIEPLGVAPT	KAKRRVVQREKRAVG	i	GAl	FLGFLGAAGSTMGA	r	S
consensus		RSELYKYKV	-KIEPLGVAPT	KAKRRVVQREKRAVG	m	-GA	-FLGFLGAAGSTMGA	-S	-TLTV
HIVJCENV	542	QARQLLSGIV	QQNNLLRAIEAQQHLLQL	TVWG	IKQLQAR	v	LAVERYLKDQQL	LGIW	GCSG
HIVlaienvt	545	QARQLLSGIV	QQNNLLRAIEAQQHLLQL	TVWG	IKQLQAR	i	LAVERYLKDQQL	LGIW	GCSG
consensus		QARQLLSGIV	QQNNLLRAIEAQQHLLQL	TVWG	IKQLQAR	-	LAVERYLKDQQL	LGIW	GCSG
HIVJCENV	603	KLIC	TTtVPWNASWSNKS	LdQIWN	NMTWm	EWDR	Ei	ANYTn	LIHh
HIVlaienvt	606	KLIC	TTaVPWNASWSNKS	L	QIWN	NMTWm	EWDR	E	i
consensus		KLIC	TT-VPWNASWSNKS	-L	-QIWN	NMTW	-EWDREI	-NYT	-LIH
HIVJCENV	664	ELDKWASL	WsfWf	I	s	NWLWYIKIFIMIV	g	LVGLRIVFAVLS	IVNRVRQGYSP
HIVlaienvt	667	ELDKWASL	WnWFn	I	t	NWLWYIKIFIMIV	g	LVGLRIVFAVLS	IVNRVRQGYSP
consensus		ELDKWASL	-W	-F	-I	-NWLWYIKIFIMIV	-g	-LVGLRIVFAVLS	-IVNRVRQGYSP
HIVJCENV	725	aPRGPDRP	dGIEgEGGERDRDRS	vRLVd	GfLALl	WeDL	RnL	CLFSYHRLRD	LLLI
HIVlaienvt	728	tPRGPDRP	eGIEeEGGERDRDRS	iRLVn	GsLALi	WdDL	RsL	CLFSYHRLRD	LLLI
consensus		-PRGPDRP	-GIE-EGGERDRDRS	-RLV	-G-LAL	-W-DLR	-LCLFSYHRLRD	LLLI	I
HIVJCENV	786	LLGRRGWE	ALKYl	W	s	LLQYWSQELKNSAV	n	Lf	n
HIVlaienvt	789	LLGRRGWE	ALKYw	W	n	LLQYWSQELKNSAV	s	L	i
consensus		LLGRRGWE	ALKY	-W	-LLQYWSQELKNSAV	-L	-N	-TAI	-VAEGTDR
HIVJCENV	847	RRIRQGLER	i	LL					
HIVlaienvt	850	RRIRQGLER	i	LL					
consensus		RRIRQGLER	-LL						

Alignment score = 657.00

Table 2C. Alignment of HIV-1<sub>JC</sub> and HIV-1<sub>sf2</sub> env Protein Sequences (SEQ ID NO:2 and SEQ ID NO:5, respectively). The consensus sequence corresponds to SEQ ID NO:8.

HIVJCENV	1	MrVKencqhlwrwgWkWGimLLGMLMICSATEKLWVTVYYGVPVWKEtTTTLFCASDAkAY
HIVsf2envt	1	MkVK gtrrnyqhlWrWGtllLLGMLMICSATEKLWVTVYYGVPVWKEaTTTLFCASDArAY
consensus		M-VKe-----W-WG--LLGMLMICSATEKLWVTVYYGVPVWKE-TTTLFCASDA-AY
HIVJCENV	62	eeEVHNVWATHACVPTDPNPQEIvLanVTEDfNMWKNmMVEQMhtDIISLWDeSLKPCVKL
HIVsf2envt	61	dteEVHNVWATHACVPTDPNPQEvVLgNVTEfNMWKNmMVEQMqeDIISLWDqSLKPCVKL
consensus		--EVHNVWATHACVPTDPNPQE-VL-NVTE-FNMWKN-MVEQM--DIISLWD-SLKPCVKL
HIVJCENV	123	TPLCVTLNCTDLkneTkTNSSdaNsnsgeimgneeikncsfinvstgagpKvqkeyalfyal
HIVsf2envt	122	TPLCVTLNCTDLgkaTnTNSS NwkeekigeikncsfnittsirdkiqKenalfnldvv
consensus		TPLCVTLNCTDL---T-TNSSdaN-----K-----
HIVJCENV	184	diVsiknenNsTshmltsCNTSVsTQACPKVSFEPIPIHYCaPAGFAILKCNdKkFNGtGP
HIVsf2envt	181	pIdnastttNyTnyrLihCNrSViTQACPKVSFEPIPIHYCtPAGFAILKCNnKtFNGkGP
consensus		-I-----N-T---L--CN-SV-TQACPKVSFEPIPIHYC-PAGFAILKCN-K-FNG-GP
HIVJCENV	245	CnNVSTVQCTHGIRPvVSTQLLNGSvAEEEVVlRSaNFsdNAKTIIVQLNhsVeItCTRP
HIVsf2envt	242	CtNVSTVQCTHGIRPiVSTQLLNGSLAEEEVViRSdNftnNAKTIIVQLNesVaInCTRP
consensus		C-NVSTVQCTHGIRP-VSTQLLNGS-AEEEVV-RS-NF--NAKTIIVQLN-SV-I-CTRP
HIVJCENV	306	NyNetKkIrIhrGygrsfvTvRklGDrkqAHctmnRtkWdNaLkQIasKLREQFnnKtaIi
HIVsf2envt	303	NnNtrKsIyIgpG rafhtTgRiiGDirkAHChisRaqWnNtLeQIvkkLREQFgNnktIv
consensus		N-N--K-I-I--Gy-----T-R--GD---AHC---R--W-N-L-QI--KLREQFgN---I-
HIVJCENV	366	FNrSSGGDIEIeMHSFNCgGEfFYCNTTtLFNsTW netTEsngkgenItLPCRIRqfvNM
HIVsf2envt	363	FNqSSGGDpEIVMHSFNCrGEfFYCNTTtLFNnTWrlnhTEgtkgndtIiLPCRiKqiINM
consensus		FN-SSGGD-EI-MHSFNC-GE-FYCNTT-LFN-TWr---TE-----I-LPCRi-Q--NM
HIVJCENV	426	WQkVGKAMYAPPsdGQIrCtSNITGLLLTRDGGhNdnNtnnEtFRPGrGDMRDNRSELYK
HIVsf2envt	424	WQeVGKAMYAPPigQIsCsSNITGLLLTRDGGtNvtN dtEvFRPGgGDMRDNRSELYK
consensus		WQ-VGKAMYAPP--GQI-C-SNITGLLLTRDGG-N--Nt--E-FRPG-GDMRDNRSELYK

Table 2C. (Continued)

HIVJCENV	487	YKVIKIEPLGvAPTKAKRRVVQREKRAVGmVGAMFLGFLGAAGSTMGAaSLTLTVQARQLL
HIVsf2envt	484	YKVIKIEPLGiAPTKAKRRVVQREKRAVGiVGAMFLGFLGAAGSTMGAvSLTLTVQARQLL
consensus		YKVIKIEPLG-APTKAKRRVVQREKRAVG-VGAMFLGFLGAAGSTMGA-SLTLTVQARQLL
HIVJCENV	548	SGIVQQQNLLRAIEAQQHLLQLTVWGIKQLQARVLAVERYLkDQQLLGIWGCSGKLICTT
HIVsf2envt	545	SGIVQQQNLLRAIEAQQHLLQLTVWGIKQLQARVLAVERYLrDQQLLGIWGCSGKLICTT
consensus		SGIVQQQNLLRAIEAQQHLLQLTVWGIKQLQARVLAVERYL-DQQLLGIWGCSGKLICTT
HIVJCENV	609	tVPWNASWSNKSldqIWnNMTWmewdREIaNYTNlihhLiEESQNQQEKNEQELLELDKWA
HIVsf2envt	606	aVPWNASWSNKSledIWdNMTWmqWeREIdNYTNtIytLlEESQNQQEKNEQELLELDKWA
consensus		-VPWNASWSNKS--IW-NMTW--W-REI-NYTN-I--L-EESQNQQEKNEQELLELDKWA
HIVJCENV	670	SLWswFdIsNWLWYIKIFIMIVaGLVGLRIVFAVLSIVNRVRQGYSPLSFQThfPaPRGPD
HIVsf2envt	667	SLWnWfsItNWLWYIKIFIMIVgGLVGLRIVFAVLSIVNRVRQGYSPLSFQTrlPvPRGPD
consensus		SLW-WF-I-NWLWYIKIFIMIV-GLVGLRIVFAVLSIVNRVRQGYSPLSFQT--P-PRGPD
HIVJCENV	731	RPDGIEgEGGERDRDRSVRLVDGFLALlWEDLRnLCLFSYhRLRDLLliVtrIvElLGrRG
HIVsf2envt	728	RPDGIEeEGGERDRDRSVRLVDGFLALiWEDLRsLCLFSYrRLRDLLLIaartVEiLghRG
consensus		RPDGIE-EGGERDRDRSVRLVDGFLAL-WEDLR-LCLFSY-RLRDLLLI--R-VE-LG-RG
HIVJCENV	792	WEALKYlWSLLQYWsQELKNSAVnlfNtTAIvVaEGTDRIIEVvQRlcrAILHIpRRIRQG
HIVsf2envt	789	WEALKYwWSLLQYWiQELKNSAVswlNaTAIaVtEGTDRvIEVaQRayRAILHIhRRIRQG
consensus		WEALKY-WSLLQYW-QELKNSAV---N-TAI-V-EGTDR-IEV-QR--RAILHI-RRIRQG
HIVJCENV	853	LERiLL
HIVsf2envt	850	LERlLL
consensus		LER-LL

Alignment score = 614.00

Table 3. HIV-1 Antibody Response of C534 After Transfusion From C455

Days Post Infection	Titer
0	0
7	0
14	400
21	1600
28	1600



Table 4. Comparative Analyses of Chimpanzee HIV-1 Isolates With Inoculating Viruses

	HIV-1 <sub>JC16</sub> vs:				Mutations unique to JC/NC <sup>2</sup>
	LAI	SF2	NDK	NC7	
<b>Pol</b>					
% homology <sup>1</sup>	96.6	94.8	94.0	98.8	-
insertions	12	0	0	0	0
deletions	0	0	1	0	0
point mutations	30	51	56	11	23
<b>Gag</b>					
% homology <sup>1</sup>	94.2	92.2	88.8	97.4	-
insertions	12	5	1	2	0
deletions	0	3	81	0	2
point mutations	25	33	42	9	20
<b>Vif</b>					
% homology <sup>1</sup>	92.2	86.0	86.5	96.9	-
insertions	0	0	0	0	0
deletions	0	0	0	0	0
point mutations	14	26	25	5	10
<b>Vpr</b>					
% homology <sup>1</sup>	88.7	96.9	89.7	96.6	-
insertions	0	0	0	0	0
deletions	1	0	1	0	0
point mutations	7	2	6	2	0
<b>Tat</b>					
% homology <sup>1</sup>	88.4	82.5	74.4	97.0	-
insertions	0	1	0	0	0
deletions	15	0	15	0	0
point mutations	10	18	22	3	4
<b>Nef</b>					
% homology <sup>1</sup>	87.4	89.4	78.7	99.0	-
insertions	0	4	1	0	0
deletions	0	0	0	0	0
point mutations	25	18	40	1	8
<b>Rev</b>					
% homology <sup>1</sup>	84.5	82.9	75.2	96.6	-
insertions	1	1	1	0	0
deletions	1	0	0	0	0
point mutations	18	19	28	4	11
<b>Env</b>					
% homology <sup>1</sup>	81.9	76.1	71.4	97.6	-
insertions	9	9	11	0	0
deletions	7	13	24	1	2
point mutations	138	173	203	17	78
<b>Vpu</b>					
% homology <sup>1</sup>	79.3	63.9	67.1	98.8	-
insertions	0	0	0	0	0
deletions	1	1	1	0	0
point mutations	13	24	24	0	10
<b>LTR</b>					
% homology <sup>1</sup>	92.1	93.9	89.1	98.7	-
insertions	1	3	1	0	0
deletions	1	3	1	0	1
point mutations	48	29	52	10	14

<sup>1</sup>Amino acid homology<sup>2</sup>Amino acids or nucleotides not observed in parental viruses<sup>3</sup>Nucleotide identity

Table 5

	-----MBD----- > matrix protein (p17)	
HIV-1JC16 gag	MGARASVLSGGDLDRWEKIRLRPGGKKKYYMLKHIVWASRELERFAVNPGLLETSEGCRCQVLEQLQPSLQT	70
HIV-1NC7 gag	.....A.....I.....	70
HIV-1LAI gag	.....K.....I.G.....	70
HIV-1SF2 gag	.....E.K.....K.....I.G.....	70
HIV-1NDK gag	.....K.T.R.....A.LI.....TL.....K.IIG.....I.....	70
	>	
HIV-1JC16 gag	GSEELRSLYNTIATLYGVHQKIEVKDTKEALDKIEEQNKSKKKAQAAA-----DTGHSQVSNYP	133
HIV-1NC7 gag	.....C.....E.....V.....AT-----G.N.....	135
HIV-1LAI gag	.....V.....C.....R.I.....	133
HIV-1SF2 gag	.....V.....C.....R.D.....E.....--AAGTON--	135
HIV-1NDK gag	.....I.....V.....C.....ER.....VE.M.....T.....	130
	capsid protein (p24)	
HIV-1JC16 gag	IVQNLCQGMVHQAI SPRTLNAWVKVIEEKAFSPVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKE	203
HIV-1NC7 gag	.....V.....	205
HIV-1LAI gag	.....I.....V.....	203
HIV-1SF2 gag	.....V.....	205
HIV-1NDK gag	.....	200
HIV-1JC16 gag	TINEEAAEWDVRVHPVQAGLIA PGQMREPRGSDIAGTTSTLQEQIGWMTSNPPIPVGEIYKRWIILGLNKI	273
HIV-1NC7 gag	.....H.P.....N.....	273
HIV-1LAI gag	.....H.P.....N.....	275
HIV-1SF2 gag	.....L.H.PV.....A.....	270
HIV-1NDK gag	.....D.....	270
HIV-1JC16 gag	VRMYSPI SILDIRQPKPEFRDYVDRFYKTLRAEQASQEVKNWMTETLLVQNANPDCKTILKALGPGATL	343
HIV-1NC7 gag	.....	345
HIV-1LAI gag	.....T.....	343
HIV-1SF2 gag	.....T.....D.....	345
HIV-1NDK gag	.....V.....D.....D.....Q.....	340
	p24 <-   -----p2----- > Nucleocapsid [ zinc finger ] (p7)	
HIV-1JC16 gag	EEMMTACQGVGGPGHKARVLAEAMSQVT-NSAAIMMQRGNFKNQKRMVKCFNCCKEGHVARNCRAPRKKG	412
HIV-1NC7 gag	.....T.....	414
HIV-1LAI gag	.....T.....R.....I.....	412
HIV-1SF2 gag	.....P.N.....R.....T.....I.K.....	414
HIV-1NDK gag	.....GSAT.V.....GP.SI.....T.K.....	410

Table 5 (continued)

	(	zinc finger	)	p7	<-	-----p1----- >	p6	
HIV-1JC16 gag	CWKCGKEGHQMKDCIESQANFLGKIWP	SHKGRPGN	FLQSRPEPTAPP	-----EESLRSGIETT	470			
HIV-1NC7 gag	.....R.....	.....	.....	.....	472			
HIV-1LA1 gag	.....T.R.....	Y.....	FLQSRPEPTAPP	.....F...V...	482			
HIV-1SF2 gag	.....R.R.....	T.R.....	Y.....	.....F.F.E.K.	472			
HIV-1NDK gag	.....R....	.....	.....	.....	420			
				p6 <-				
HIV-1JC16 gag	TPSQKQEPIDKEVYPLTSLRSLFGNDPSSQ				501			
HIV-1NC7 gag	.....	.....	.....		503			
HIV-1LA1 gag	.....L.....	.....	.....		513			
HIV-1SF2 gag	.....L.....	.....	.....		503			
HIV-1NDK gag	.....	.....	.....		420			

Table 5 (continued)

--MTD---	
HIV-1JC16 nef	MGKWSKSSIVGWP TIRERMKRAG---PAADGVGAASRDLEKKGAI TSSNTAATNADCA 56
HIV-1NC7 nef	.....V.....R.....R..... 56
HIV-1LAI nef	.....V.....R.....E.....A..... 56
HIV-1SF2 nef	.....R.MG..SA.....R..EPRAE.....V..... 60
HIV-1NDK nef	.....L.....A.....IRKTD.....V.....S..DT.. 56
LCK binding domain	
-----	
HIV-1JC16 nef	WLEAQEE-EEVGFPVRPQVPLRPMTYKAGIDL SHFLKEKGGLEGLVWSQRRQDILD LWIY 115
HIV-1NC7 nef	.....T.....AV.....IH..... 115
HIV-1LAI nef	.....S.....AL.I.....I.....E..... 119
HIV-1SF2 nef	.....S.....EAV.....I..KK..E.....V. 116
HIV-1JC16 nef	HTQGYFPDWNQYTPGPGIRYPLTFGWCFKLVPEPKVVEANEGEDNILLHPMCLHGMED 175
HIV-1NC7 nef	.....V.....Y.....K..NTS....VS....D. 175
HIV-1LAI nef	.....I.....E.....N.S.....S..... 179
HIV-1SF2 nef	N..I.....Q.....D.QE....T.R..C.....QQ.... 176
HIV-1NDK nef	AEKEVLVWRFD SKLAFHHVARELHPEYKDC 207
HIV-1NC7 nef	.....P.R..E.....R.....F.N. 207
HIV-1LAI nef	.....M..... 211
HIV-1SF2 nef	P.RQ..M...N.R..LE.K.....F.... 208

signal peptide <-|->gp120 (SU)

HIV-1JC16	env	MVKE--NCQHLWRGWKGIMLLGLMLICSATEKLWTVVYGVPUVKETTTTLFCASD	57
HIV-1NC7	env	--Y.	57
HIV-1LAI	env	--KY..T..I.	57
HIV-1SF2	env	.K.GTRR.Y.--TL.	56
HIV-1NDK	env	.AR.KER.N.....T.A.D.....I.A.	56
HIV-1JC16	env	AKAYEEVHNWATHACVPTDPNPQEIVLANVTEDFNMWKNEVMVEQMHTDIISLWDESLK	117
HIV-1NC7	env	DT..V.V.N..D..E..Q..	117
HIV-1LAI	env	R.DT..V.G.N..N.QE.Q..	116
HIV-1SF2	env	.KK.A.I.....E.E.N..N..E..Q..	116
HIV-1NDK	env	PCVKLTPLCVTLNCTDLKNETKTNSDANSNGEIM-GNEEIKNCSFNSTGAPKVQKE	176
HIV-1JC16	env	S.K..G.A.N..NT.S..M.MEKG..I..SIR..	177
HIV-1LAI	env	GKA.N..NWKEE-----KG...IT.SIRD.I..	170
HIV-1SF2	env	ELRNS.G.GK-----VEEE.KR....--RD.REQV	163
HIV-1NDK	env	YALFYALDIVSIKENNSTSH--MLTSCNTSVSTQACPVSFEPIPIHYCAPAGFAILK	233
HIV-1NC7	env	F.K.K..IP.D.DTT-----YT..I..	232
HIV-1LAI	env	N..RN.V.P.D.ASTT.NYTNYR.IH.R.I..T..	230
HIV-1SF2	env	K..P.D.N.RTN.-TNYR.IN.D.TI..I..F..	222
HIV-1NDK	env	CNDKKFNGTGPCNNVSTVQCTHGIRPVVSTQLLNGSVAEEEEVLRSANFSDNAKTIIVQ	293
HIV-1JC16	env	N.T..T..L..L..I..T..	293
HIV-1LAI	env	N.T..K..T..I..L..I.D.TN..	290
HIV-1SF2	env	R..S.....L..III.E.LTN.V..	282
HIV-1NDK	env	LNHSEITCRPNYNETKKIRIHRYGRSFVTV--RKLGDRKQAHCMTMNRTKWDNALK	349
HIV-1NC7	env	Q..N..N.TR.S.Q..P.A..IG--KI-NMR..NIS.A..NAT..	348
HIV-1LAI	env	E.A.N..N.TR.S.Y..P.A.H.TG--II.IRK..NIS.AQ.N.T.E	345
HIV-1SF2	env	A.IV.N..YKYTRQRTS--LRQ..LY.ITGKKK.T.YIG...KIS.AB.NK..Q	340
HIV-1NDK	env		

Table 5 (continued)

HIV-1JC16 env	QIASKLREQFNKTAIIFNRSSGGDLEIEMHSFNCGGELFYCNTTKLFNSTW	-----V4-----	NE	403
HIV-1NC7 env	.....	-----V4-----	NE	402
HIV-1LAI env	.....G.NKT...K.Q...P.VT...F...S.Q...FNSTWST	-----V4-----	NE	408
HIV-1SF2 env	..VK...G.NKT.V.Q...P.V...R.F...Q.N...WRLN	-----V4-----	NE	400
HIV-1NDK env	.V.T.-GNLL...T.T.KP...P.TS.ML...DF...SR...Q	-----V4-----	NE	393
CD4 binding				
	-----V4----- -----V5-----			
HIV-1JC16 env	TTESNGKGENITLPCRIRQFVNMQKVGKAMYAPPSDQIRCTSNITGLLLTRDGGHNDN	-----V4----- -----V5-----		463
HIV-1NC7 env	.....	-----V4----- -----V5-----		462
HIV-1LAI env	GSNNTEGSDT...K.I...E...IS...S...N..	-----V4----- -----V5-----		467
HIV-1SF2 env	H.GTKGNDT.I...K.II...E...IG...S.S...T.VT	-----V4----- -----V5-----		460
HIV-1NDK env	.NSTGFNNGTV...K.I..L.R...IE.L.K.S...A..	-----V4----- -----V5-----		451
	---V5---			
HIV-1JC16 env	NTNNETFRPGRGDMRDNRSELYKVKYKIEPLGVAPTAKRRVVQREKRAVGMVGAMFL	gp120 (SU) <- -> gp41 (TM)		523
HIV-1NC7 env	.KTDK...G...V...I...L..			522
HIV-1LAI env	..GS.I...G...V...I...L..			525
HIV-1SF2 env	..DT.V...G...I...I...V...I..			519
HIV-1NDK env	.SSH..I...G...V...I...R...E...I.L..V..			510
HIV-1JC16 env	GFLGAAGSTMGAASLTLTVQARQLLSGIVQQNNLLRAIEAQHLLQLTVWGIKQLQARV			583
HIV-1NC7 env	.....			582
HIV-1LAI env	.....R.M...I...I...I...I			585
HIV-1SF2 env	.....V...M...H...I...I...I...I			579
HIV-1NDK env	.....V...M...H...I...I...I...I			570
HIV-1JC16 env	LAVERYLKDQQLLGWCSGKLICTTVPWNASWSNKSLSLDQIWNNTWMEWDREIANYTN			643
HIV-1NC7 env	.....L...L...L...L...L...L			642
HIV-1LAI env	.....A...A...A...A...A...A...N...S			645
HIV-1SF2 env	.....R...A...A...A...A...A...Q.E...D...G			639
HIV-1NDK env	.....R...RH...N...S...R...E...Q...E...D...G			630
HIV-1JC16 env	LIHHLIEESQNOQEKNEQELLELDKWSLWSWFDISNWLWYKIFIMIVAGLVGLRIVFA			703
HIV-1NC7 env	.....			702
HIV-1LAI env	.....S...N...N...T...G...G...G...G			705
HIV-1SF2 env	T.YT.L...N...N...S...T...G...G...G...G			699
HIV-1NDK env	.YS...I...K...N...S...TK...L...G...I...I			690

Table 5 (continued)

HIV-1JC16 env	VLSIVNRVQGYSPLSFQTHFPAPRGPD	RDGIEGEGGERDRDRSVRLVDGFLALLWEDL	763
HIV-1NC7 env	.....	.....	762
HIV-1LAI env	.....	.....	765
HIV-1SF2 env	.....	.....	759
HIV-1NDK env	.....	.....	750
HIV-1JC16 env	RNLCCLFSYHRLRDLLIVTRIVELLGRRGWE	ALKYLSLLQYWSQELKNSAVNLFNTTAI	823
HIV-1NC7 env	.....	.....	822
HIV-1LAI env	.....	.....	825
HIV-1SF2 env	.....	.....	819
HIV-1NDK env	.....	.....	810
HIV-1JC16 env	VVAEGTDRIIEVQRLCRAILHIPRRIRQGLERILL		860
HIV-1NC7 env	.....	.....	859
HIV-1LAI env	.....	.....	862
HIV-1SF2 env	.....	.....	856
HIV-1NDK env	.....	.....	847

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: EMORY UNIVERSITY
- (ii) TITLE OF INVENTION: Human Immunodeficiency Viruses  
Causing AIDS in a Nonhuman Primate
- (iii) NUMBER OF SEQUENCES: 23
- (iv) CORRESPONDENCE ADDRESS:
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  - (E) COUNTRY: US
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- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: WO
  - (B) FILING DATE: 23-JUN-1998
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 60/050,548
  - (B) FILING DATE: 23-JUN-1997
- (viii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 60/057,606
  - (B) FILING DATE: 04-SEP-1997
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## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2577 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: not relevant



(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..2577

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG AGA GTG AAG GAG AAC TGT CAG CAC TTG TGG AGA TGG GGG TGG AAA	48
Met Arg Val Lys Glu Asn Cys Gln His Leu Trp Arg Trp Gly Trp Lys	
1 5 10 15	
TGG GGC ATC ATG CTC CTT GGG ATG TTA ATG ATC TGT AGT GCT ACA GAA	96
Trp Gly Ile Met Leu Leu Gly Met Leu Met Ile Cys Ser Ala Thr Glu	
20 25 30	
AAA TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT GTG TGG AAG GAA ACA	144
Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Thr	
35 40 45	
ACT ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA GCA TAT GAA GAA GAG	192
Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Glu Glu Glu	
50 55 60	
GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA CCC ACA GAC CCC AAC	240
Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn	
65 70 75 80	
CCA CAA GAA ATA GTA TTG GCA AAT GTG ACA GAA GAT TTT AAC ATG TGG	288
Pro Gln Glu Ile Val Leu Ala Asn Val Thr Glu Asp Phe Asn Met Trp	
85 90 95	
AAA AAT GAA ATG GTA GAA CAG ATG CAT ACT GAT ATA ATC AGT TTA TGG	336
Lys Asn Glu Met Val Glu Gln Met His Thr Asp Ile Ile Ser Leu Trp	
100 105 110	
GAT GAA AGC CTA AAA CCA TGT GTA AAA TTA ACC CCA CTC TGT GTT ACT	384
Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr	
115 120 125	
TTA AAT TGC ACT GAT TTG AAG AAT GAA ACT AAG ACC AAT AGT AGT GAT	432
Leu Asn Cys Thr Asp Leu Lys Asn Glu Thr Lys Thr Asn Ser Ser Asp	
130 135 140	
GCC AAT AGT AAT AGC GGG GAA ATA ATG GGG AAC GAA GAG ATA AAA AAT	480
Ala Asn Ser Asn Ser Gly Glu Ile Met Gly Asn Glu Glu Ile Lys Asn	
145 150 155 160	
TGC TCT TTC AAT GTC AGC ACA GGC GCA CCA GGT AAG GTG CAG AAA GAA	528
Cys Ser Phe Asn Val Ser Thr Gly Ala Pro Gly Lys Val Gln Lys Glu	
165 170 175	

TAT TCA CTT TTT TAT GCA CTT GAT ATA GTA TCA ATA AAG AAT GAA AAT	576
Tyr Ser Leu Phe Tyr Ala Leu Asp Ile Val Ser Ile Lys Asn Glu Asn	
180 185 190	
AAT AGT ACC AGC CAT ATG TTG ACA AGT TGT AAC ACC TCA GTC AGT ACA	624
Asn Ser Thr Ser His Met Leu Thr Ser Cys Asn Thr Ser Val Ser Thr	
195 200 205	
CAG GCC TGT CCA AAG GTA TCC TTT GAG CCA ATT CCC ATA CAT TAT TGT	672
Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro Ile His Tyr Cys	
210 215 220	
GCC CCG GCT GGT TTT GCA ATT CTA AAA TGT AAT GAT AAG AAG TTC AAT	720
Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Lys Phe Asn	
225 230 235 240	
GGA ACA GGA CCA TGT AAC AAT GTC AGC ACA GTA CAA TGT ACA CAT GGA	768
Gly Thr Gly Pro Cys Asn Asn Val Ser Thr Val Gln Cys Thr His Gly	
245 250 255	
ATT AGA CCA GTA GTG TCA ACT CAA CTG CTG TTA AAT GGC AGT GTA GCA	816
Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Val Ala	
260 265 270	
GAA GAA GAG GTA GTA CTT AGA TCT GCC AAT TTC TCA GAC AAT GCT AAA	864
Glu Glu Glu Val Val Leu Arg Ser Ala Asn Phe Ser Asp Asn Ala Lys	
275 280 285	
ACC ATA ATA GTA CAG CTG AAC CAC TCT GTA GAA ATT ACT TGT ACA AGA	912
Thr Ile Ile Val Gln Leu Asn His Ser Val Glu Ile Thr Cys Thr Arg	
290 295 300	
CCC AAC TAC AAT GAA ACA AAG AAA ATC CGT ATC CAC AGA GGA TAT GGA	960
Pro Asn Tyr Asn Glu Thr Lys Lys Ile Arg Ile His Arg Gly Tyr Gly	
305 310 315 320	
AGA TCA TTT GTT ACA GTA AGA AAA TTG GGA GAT AGG AAA CAA GCA CAT	1008
Arg Ser Phe Val Thr Val Arg Lys Leu Gly Asp Arg Lys Gln Ala His	
325 330 335	
TGT ACC ATG AAT AGA ACG AAA TGG GAC AAC GCT TTA AAA CAG ATA GCT	1056
Cys Thr Met Asn Arg Thr Lys Trp Asp Asn Ala Leu Lys Gln Ile Ala	
340 345 350	
AGC AAA TTA AGA GAA CAA TTT AAT AAA ACA GCA ATA ATC TTT AAC CGG	1104
Ser Lys Leu Arg Glu Gln Phe Asn Lys Thr Ala Ile Ile Phe Asn Arg	
355 360 365	
TCC TCA GGA GGG GAC CTA GAA ATT GAA ATG CAC AGT TTT AAT TGC GGA	1152
Ser Ser Gly Gly Asp Leu Glu Ile Glu Met His Ser Phe Asn Cys Gly	
370 375 380	
GGG GAA TTG TTC TAC TGT AAT ACA ACA AAA CTG TTT AAT AGT ACT TGG	1200
Gly Glu Leu Phe Tyr Cys Asn Thr Thr Lys Leu Phe Asn Ser Thr Trp	
385 390 395 400	

AAT GAG ACT ACA GAG TCA AAT GGC AAG GGA GAA AAT ATC ACA CTC CCA Asn Glu Thr Thr Glu Ser Asn Gly Lys Gly Glu Asn Ile Thr Leu Pro 405 410 415	1248
TGC AGA ATA AGA CAA TTT GTA AAC ATG TGG CAG AAA GTA GGA AAA GCA Cys Arg Ile Arg Gln Phe Val Asn Met Trp Gln Lys Val Gly Lys Ala 420 425 430	1296
ATG TAT GCC CCT CCC AGC GAT GGA CAA ATT AGG TGT ACA TCA AAT ATT Met Tyr Ala Pro Pro Ser Asp Gly Gln Ile Arg Cys Thr Ser Asn Ile 435 440 445	1344
ACT GGG CTA CTA TTA ACA AGA GAT GGG GGT CAT AAT GAT AAC AAC ACT Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly His Asn Asp Asn Asn Thr 450 455 460	1392
AAC AAC GAG ACC TTC AGA CCG GGA AGA GGA GAT ATG AGG GAC AAT TGG Asn Asn Glu Thr Phe Arg Pro Gly Arg Gly Asp Met Arg Asp Asn Trp 465 470 475 480	1440
AGA AGT GAA TTA TAT AAA TAT AAA GTA ATA AAA ATT GAA CCA TTA GGA Arg Ser Glu Leu Tyr Lys Tyr Lys Val Ile Lys Ile Glu Pro Leu Gly 485 490 495	1488
GTA GCA CCC ACC AAG GCA AAG AGA AGA GTG GTG CAG AGA GAA AAA AGA Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg 500 505 510	1536
GCA GTG GGA ATG GTA GGA GCT ATG TTC CTT GGG TTC TTG GGA GCA GCA Ala Val Gly Met Val Gly Ala Met Phe Leu Gly Phe Leu Gly Ala Ala 515 520 525	1584
GGA AGC ACT ATG GGC GCA GCG TCA TTG ACG CTG ACG GTA CAG GCC AGA Gly Ser Thr Met Gly Ala Ala Ser Leu Thr Leu Thr Val Gln Ala Arg 530 535 540	1632
CAA TTA TTG TCT GGT ATA GTG CAG CAG CAG AAC AAT CTG CTG AGA GCT Gln Leu Leu Ser Gly Ile Val Gln Gln Gln Asn Asn Leu Leu Arg Ala 545 550 555 560	1680
ATT GAG GCG CAA CAA CAT CTG TTG CAA CTC ACA GTC TGG GGC ATC AAG Ile Glu Ala Gln Gln His Leu Leu Gln Leu Thr Val Trp Gly Ile Lys 565 570 575	1728
CAG CTC CAG GCA AGA GTC CTG GCT GTA GAA AGA TAC CTA AAG GAT CAA Gln Leu Gln Ala Arg Val Leu Ala Val Glu Arg Tyr Leu Lys Asp Gln 580 585 590	1776
CAG CTC CTG GGG ATC TGG GGT TGC TCT GGA AAA CTC ATT TGC ACC ACT Gln Leu Leu Gly Ile Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr 595 600 605	1824
ACT GTG CCT TGG AAT GCT AGT TGG AGT AAT AAA TCT TTG GAT CAG ATT Thr Val Pro Trp Asn Ala Ser Trp Ser Asn Lys Ser Leu Asp Gln Ile 610 615 620	1872

TGG AAT AAC ATG ACC TGG TTG GAG TGG GAC AGA GAA ATT GCC AAT TAC Trp Asn Asn Met Thr Trp Leu Glu Trp Asp Arg Glu Ile Ala Asn Tyr 625 630 635 640	1920
ACA AAC TTA ATA CAT CAC TTA ATT GAA GAA TCG CAA AAC CAG CAA GAA Thr Asn Leu Ile His His Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu 645 650 655	1968
AAG AAT GAA CAA GAA TTA TTG GAA TTA GAT AAA TGG GCA AGT TTG TGG Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp 660 665 670	2016
AGT TGG TTT GAC ATA TCA AAC TGG CTG TGG TAT ATA AAA ATA TTC ATA Ser Trp Phe Asp Ile Ser Asn Trp Leu Trp Tyr Ile Lys Ile Phe Ile 675 680 685	2064
ATG ATA GTA GCA GGC TTA GTA GGT TTA AGA ATA GTT TTT GCT GTG CTT Met Ile Val Ala Gly Leu Val Gly Leu Arg Ile Val Phe Ala Val Leu 690 695 700	2112
TCT ATA GTA AAT AGA GTT AGG CAG GGA TAC TCA CCA TTG TCA TTC CAG Ser Ile Val Asn Arg Val Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln 705 710 715 720	2160
ACC CAC TTC CCA GCT CCG AGG GGA CCC GAC AGG CCA GAC GGA ATC GAA Thr His Phe Pro Ala Pro Arg Gly Pro Asp Arg Pro Asp Gly Ile Glu 725 730 735	2208
GGA GAA GGT GGA GAG AGA GAC AGA GAC AGA TCC GTG CGA TTA GTG GAT Gly Glu Gly Gly Glu Arg Asp Arg Asp Arg Ser Val Arg Leu Val Asp 740 745 750	2256
GGA TTC TTA GCA CTT CTC TGG GAA GAC CTG CGC AAC CTG TGC CTC TTC Gly Phe Leu Ala Leu Leu Trp Glu Asp Leu Arg Asn Leu Cys Leu Phe 755 760 765	2304
AGC TAC CAC CGC TTG AGA GAC TTA CTC TTG ATT GTA ACG AGG ATT GTG Ser Tyr His Arg Leu Arg Asp Leu Leu Leu Ile Val Thr Arg Ile Val 770 775 780	2352
GAA CTT CTC GGA CGC AGG GGG TGG GAA GCC CTC AAA TAT TTG TGG AGT Glu Leu Leu Gly Arg Arg Gly Trp Glu Ala Leu Lys Tyr Leu Trp Ser 785 790 795 800	2400
CTC CTA CAG TAT TGG AGT CAG GAG CTA AAG AAT AGT GCT GTC AAC TTG Leu Leu Gln Tyr Trp Ser Gln Glu Leu Lys Asn Ser Ala Val Asn Leu 805 810 815	2448
TTC AAT ACC ACA GCT ATA GTA GTA GCT GAG GGG ACA GAT AGG ATC ATA Phe Asn Thr Thr Ala Ile Val Val Ala Glu Gly Thr Asp Arg Ile Ile 820 825 830	2496
GAA GTA GTA CAA AGA CTT TGT AGA GCT ATT CTC CAC ATA CCT AGA AGA Glu Val Val Gln Arg Leu Cys Arg Ala Ile Leu His Ile Pro Arg Arg 835 840 845	2544

ATT AGA CAG GGC TTG GAA AGA TTT TTG CTA TAA  
 Ile Arg Gln Gly Leu Glu Arg Phe Leu Leu \*  
 850 855

2577

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 859 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Arg Val Lys Glu Asn Cys Gln His Leu Trp Arg Trp Gly Trp Lys  
 1 5 10 15  
 Trp Gly Ile Met Leu Leu Gly Met Leu Met Ile Cys Ser Ala Thr Glu  
 20 25 30  
 Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Thr  
 35 40 45  
 Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Glu Glu Glu  
 50 55 60  
 Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn  
 65 70 75 80  
 Pro Gln Glu Ile Val Leu Ala Asn Val Thr Glu Asp Phe Asn Met Trp  
 85 90 95  
 Lys Asn Glu Met Val Glu Gln Met His Thr Asp Ile Ile Ser Leu Trp  
 100 105 110  
 Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr  
 115 120 125  
 Leu Asn Cys Thr Asp Leu Lys Asn Glu Thr Lys Thr Asn Ser Ser Asp  
 130 135 140  
 Ala Asn Ser Asn Ser Gly Glu Ile Met Gly Asn Glu Glu Ile Lys Asn  
 145 150 155 160  
 Cys Ser Phe Asn Val Ser Thr Gly Ala Pro Gly Lys Val Gln Lys Glu  
 165 170 175  
 Tyr Ser Leu Phe Tyr Ala Leu Asp Ile Val Ser Ile Lys Asn Glu Asn  
 180 185 190  
 Asn Ser Thr Ser His Met Leu Thr Ser Cys Asn Thr Ser Val Ser Thr  
 195 200 205  
 Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro Ile His Tyr Cys  
 210 215 220

Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Lys Phe Asn  
 225 230 235 240  
 Gly Thr Gly Pro Cys Asn Asn Val Ser Thr Val Gln Cys Thr His Gly  
 245 250 255  
 Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Val Ala  
 260 265 270  
 Glu Glu Glu Val Val Leu Arg Ser Ala Asn Phe Ser Asp Asn Ala Lys  
 275 280 285  
 Thr Ile Ile Val Gln Leu Asn His Ser Val Glu Ile Thr Cys Thr Arg  
 290 295 300  
 Pro Asn Tyr Asn Glu Thr Lys Lys Ile Arg Ile His Arg Gly Tyr Gly  
 305 310 315 320  
 Arg Ser Phe Val Thr Val Arg Lys Leu Gly Asp Arg Lys Gln Ala His  
 325 330 335  
 Cys Thr Met Asn Arg Thr Lys Trp Asp Asn Ala Leu Lys Gln Ile Ala  
 340 345 350  
 Ser Lys Leu Arg Glu Gln Phe Asn Lys Thr Ala Ile Ile Phe Asn Arg  
 355 360 365  
 Ser Ser Gly Gly Asp Leu Glu Ile Glu Met His Ser Phe Asn Cys Gly  
 370 375 380  
 Gly Glu Leu Phe Tyr Cys Asn Thr Thr Lys Leu Phe Asn Ser Thr Trp  
 385 390 395 400  
 Asn Glu Thr Thr Glu Ser Asn Gly Lys Gly Glu Asn Ile Thr Leu Pro  
 405 410 415  
 Cys Arg Ile Arg Gln Phe Val Asn Met Trp Gln Lys Val Gly Lys Ala  
 420 425 430  
 Met Tyr Ala Pro Pro Ser Asp Gly Gln Ile Arg Cys Thr Ser Asn Ile  
 435 440 445  
 Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly His Asn Asp Asn Asn Thr  
 450 455 460  
 Asn Asn Glu Thr Phe Arg Pro Gly Arg Gly Asp Met Arg Asp Asn Trp  
 465 470 475 480  
 Arg Ser Glu Leu Tyr Lys Tyr Lys Val Ile Lys Ile Glu Pro Leu Gly  
 485 490 495  
 Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg  
 500 505 510  
 Ala Val Gly Met Val Gly Ala Met Phe Leu Gly Phe Leu Gly Ala Ala  
 515 520 525

Gly Ser Thr Met Gly Ala Ala Ser Leu Thr Leu Thr Val Gln Ala Arg  
 530 535 540  
 Gln Leu Leu Ser Gly Ile Val Gln Gln Gln Asn Asn Leu Leu Arg Ala  
 545 550 555 560  
 Ile Glu Ala Gln Gln His Leu Leu Gln Leu Thr Val Trp Gly Ile Lys  
 565 570 575  
 Gln Leu Gln Ala Arg Val Leu Ala Val Glu Arg Tyr Leu Lys Asp Gln  
 580 585 590  
 Gln Leu Leu Gly Ile Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr  
 595 600 605  
 Thr Val Pro Trp Asn Ala Ser Trp Ser Asn Lys Ser Leu Asp Gln Ile  
 610 615 620  
 Trp Asn Asn Met Thr Trp Leu Glu Trp Asp Arg Glu Ile Ala Asn Tyr  
 625 630 635 640  
 Thr Asn Leu Ile His His Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu  
 645 650 655  
 Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp  
 660 665 670  
 Ser Trp Phe Asp Ile Ser Asn Trp Leu Trp Tyr Ile Lys Ile Phe Ile  
 675 680 685  
 Met Ile Val Ala Gly Leu Val Gly Leu Arg Ile Val Phe Ala Val Leu  
 690 695 700  
 Ser Ile Val Asn Arg Val Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln  
 705 710 715 720  
 Thr His Phe Pro Ala Pro Arg Gly Pro Asp Arg Pro Asp Gly Ile Glu  
 725 730 735  
 Gly Glu Gly Gly Glu Arg Asp Arg Asp Arg Ser Val Arg Leu Val Asp  
 740 745 750  
 Gly Phe Leu Ala Leu Leu Trp Glu Asp Leu Arg Asn Leu Cys Leu Phe  
 755 760 765  
 Ser Tyr His Arg Leu Arg Asp Leu Leu Leu Ile Val Thr Arg Ile Val  
 770 775 780  
 Glu Leu Leu Gly Arg Arg Gly Trp Glu Ala Leu Lys Tyr Leu Trp Ser  
 785 790 795 800  
 Leu Leu Gln Tyr Trp Ser Gln Glu Leu Lys Asn Ser Ala Val Asn Leu  
 805 810 815  
 Phe Asn Thr Thr Ala Ile Val Val Ala Glu Gly Thr Asp Arg Ile Ile  
 820 825 830

Glu Val Val Gln Arg Leu Cys Arg Ala Ile Leu His Ile Pro Arg Arg  
 835 840 845

Ile Arg Gln Gly Leu Glu Arg Phe Leu Leu \*  
 850 855

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 854 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Arg Ala Arg Glu Lys Glu Arg Asn Cys Gln Asn Leu Trp Lys Trp  
 1 5 10 15

Gly Ile Met Leu Leu Gly Met Leu Met Thr Cys Ser Ala Ala Glu Asp  
 20 25 30

Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Ile Trp Lys Glu Ala Thr  
 35 40 45

Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Lys Lys Glu Ala  
 50 55 60

His Asn Ile Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro  
 65 70 75 80

Gln Glu Ile Glu Leu Glu Asn Val Thr Glu Asn Phe Asn Met Trp Lys  
 85 90 95

Asn Asn Met Val Glu Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp  
 100 105 110

Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu  
 115 120 125

Asn Cys Thr Asp Leu Lys Asn Glu Thr Lys Thr Asn Ser Ser Asp Ala  
 130 135 140

Asn Ser Asn Ser Gly Glu Ile Met Gly Asn Glu Glu Ile Lys Asn Cys  
 145 150 155 160

Ser Phe Asn Val Ser Thr Gly Ala Pro Gly Lys Val Gln Lys Glu Tyr  
 165 170 175

Ser Leu Phe Tyr Ala Leu Asp Asn Asn Asn Arg Thr Asn Ser Thr Asn  
 180 185 190



Tyr Arg Leu Ile Asn Cys Asp Thr Ser Thr Ile Thr Gln Ala Cys Pro  
 195 200 205  
 Lys Ile Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly  
 210 215 220  
 Phe Ala Ile Leu Lys Cys Arg Asp Lys Lys Phe Asn Gly Thr Gly Pro  
 225 230 235 240  
 Cys Ser Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val  
 245 250 255  
 Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Ile  
 260 265 270  
 Ile Ile Arg Ser Glu Asn Leu Thr Asn Asn Val Lys Thr Ile Ile Val  
 275 280 285  
 Gln Leu Asn Ala Ser Ile Val Ile Asn Cys Thr Arg Pro Tyr Lys Tyr  
 290 295 300  
 Thr Arg Gln Arg Thr Ser Ile Gly Leu Arg Gln Ser Leu Tyr Thr Ile  
 305 310 315 320  
 Thr Gly Lys Lys Lys Lys Thr Gly Tyr Ile Gly Gln Ala His Cys Lys  
 325 330 335  
 Ile Ser Arg Ala Glu Trp Asn Lys Ala Leu Gln Gln Val Ala Thr Lys  
 340 345 350  
 Leu Gly Asn Leu Leu Asn Lys Thr Thr Ile Thr Phe Lys Pro Ser Ser  
 355 360 365  
 Gly Gly Asp Pro Glu Ile Thr Ser His Met Leu Asn Cys Gly Gly Asp  
 370 375 380  
 Phe Phe Tyr Cys Asn Thr Ser Arg Leu Phe Asn Ser Thr Trp Asn Gln  
 385 390 395 400  
 Thr Asn Ser Thr Gly Phe Asn Asn Gly Thr Val Thr Leu Pro Cys Arg  
 405 410 415  
 Ile Lys Gln Ile Val Asn Leu Trp Gln Arg Val Gly Lys Ala Met Tyr  
 420 425 430  
 Ala Pro Pro Ile Glu Gly Leu Ile Lys Cys Ser Ser Asn Ile Thr Gly  
 435 440 445  
 Leu Leu Leu Thr Arg Asp Gly Gly Ala Asn Asn Ser Ser His Glu Thr  
 450 455 460  
 Ile Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu  
 465 470 475 480  
 Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Ile Gly Val Ala Pro Thr  
 485 490 495

Lys Ala Arg Arg Arg Val Val Glu Arg Glu Lys Arg Ala Ile Gly Leu  
 500 505 510  
 Gly Ala Val Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly  
 515 520 525  
 Ala Ala Ser Val Thr Leu Thr Val Gln Ala Arg Gln Leu Met Ser Gly  
 530 535 540  
 Ile Val His Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln  
 545 550 555 560  
 His Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg  
 565 570 575  
 Val Leu Ala Val Glu Arg Tyr Leu Arg Asp Gln Gln Leu Leu Gly Ile  
 580 585 590  
 Trp Gly Cys Ser Gly Arg His Ile Cys Thr Thr Asn Val Pro Trp Asn  
 595 600 605  
 Ser Ser Trp Ser Asn Arg Ser Leu Asp Glu Ile Trp Gln Asn Met Thr  
 610 615 620  
 Trp Met Glu Trp Glu Arg Glu Ile Asp Asn Tyr Thr Gly Leu Ile Tyr  
 625 630 635 640  
 Ser Leu Ile Glu Glu Ser Gln Ile Gln Gln Glu Lys Asn Glu Lys Glu  
 645 650 655  
 Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe Ser Ile  
 660 665 670  
 Thr Lys Trp Leu Trp Tyr Ile Lys Leu Phe Ile Met Ile Val Gly Gly  
 675 680 685  
 Leu Ile Gly Leu Arg Ile Val Phe Ala Val Leu Ser Val Val Asn Arg  
 690 695 700  
 Val Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln Thr Leu Leu Pro Val  
 705 710 715 720  
 Pro Arg Gly Pro Asp Arg Pro Glu Glu Ile Glu Glu Glu Gly Gly Glu  
 725 730 735  
 Arg Gly Arg Asp Arg Ser Ile Arg Leu Val Asn Gly Leu Phe Ala Leu  
 740 745 750  
 Phe Trp Asp Asp Leu Arg Asn Leu Cys Leu Phe Ser Tyr His Arg Leu  
 755 760 765  
 Arg Asp Ser Ile Leu Ile Ala Ala Arg Ile Val Glu Leu Leu Gly Arg  
 770 775 780  
 Arg Gly Trp Glu Ala Leu Lys Tyr Leu Trp Asn Leu Leu Gln Tyr Trp  
 785 790 795 800

Ser Gln Glu Leu Arg Asn Ser Ala Ser Ser Leu Leu Asp Thr Ile Ala  
805 810 815

Ile Ala Val Ala Glu Arg Thr Asp Arg Val Ile Glu Val Val Gln Arg  
820 825 830

Ala Cys Arg Ala Ile Leu Asn Val Pro Arg Arg Ile Arg Gln Gly Leu  
835 840 845

Glu Arg Leu Leu Leu Xaa  
850

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 862 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Arg Val Lys Glu Lys Tyr Gln His Leu Trp Arg Trp Gly Trp Lys  
1 5 10 15

Trp Gly Thr Met Leu Leu Gly Ile Leu Met Ile Cys Ser Ala Thr Glu  
20 25 30

Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala  
35 40 45

Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu  
50 55 60

Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn  
65 70 75 80

Pro Gln Glu Val Val Leu Val Asn Val Thr Glu Asn Phe Asn Met Trp  
85 90 95

Lys Asn Asp Met Val Glu Gln Met His Glu Asp Ile Ile Ser Leu Trp  
100 105 110

Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Ser  
115 120 125

Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn Thr Asn Ser Ser Asn  
130 135 140

Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu Lys Gly Glu Ile Lys  
145 150 155 160

Asn Cys Ser Phe	Asn Ile Ser Thr	Ser Ile Arg Gly Lys Val	Gln Lys
165	170		175
Glu Tyr Ala Phe	Phe Tyr Lys Leu Asp	Ile Ile Pro Ile Asp	Asn Asp
180	185		190
Thr Thr Ser Tyr	Thr Leu Thr Ser Cys	Asn Thr Ser Val	Ile Thr Gln
195	200		205
Ala Cys Pro Lys	Val Ser Phe Glu Pro	Ile Pro Ile His	Tyr Cys Ala
210	215		220
Pro Ala Gly Phe	Ala Ile Leu Lys Cys	Asn Asn Lys Thr	Phe Asn Gly
225	230		235
Thr Gly Pro Cys	Thr Asn Val Ser Thr	Val Gln Cys Thr	His Gly Ile
	245		250
Arg Pro Val Val	Ser Thr Gln Leu Leu	Leu Asn Gly Ser	Leu Ala Glu
	260		265
Glu Glu Val Val	Ile Arg Ser Ala Asn	Phe Thr Asp Asn	Ala Lys Thr
	275		280
Ile Ile Val Gln	Leu Asn Gln Ser	Val Glu Ile Asn	Cys Thr Arg Pro
	290		295
Asn Asn Asn Thr	Arg Lys Ser Ile Arg	Ile Gln Arg Gly	Pro Gly Arg
	305		310
Ala Phe Val Thr	Ile Gly Lys Ile Gly	Asn Met Arg Gln	Ala His Cys
	325		330
Asn Ile Ser Arg	Ala Lys Trp Asn	Ala Thr Leu Lys	Gln Ile Ala Ser
	340		345
Lys Leu Arg Glu	Gln Phe Gly Asn	Asn Lys Thr Ile	Ile Phe Lys Gln
	355		360
Ser Ser Gly Gly	Asp Pro Glu Ile	Val Thr His Ser	Phe Asn Cys Gly
	370		375
Gly Glu Phe Phe	Tyr Cys Asn Ser Thr	Gln Leu Phe Asn	Ser Thr Trp
	385		390
Phe Asn Ser Thr	Trp Ser Thr Glu	Gly Ser Asn Asn	Thr Glu Gly Ser
	405		410
Asp Thr Ile Thr	Leu Pro Cys Arg Ile	Lys Gln Phe Ile	Asn Met Trp
	420		425
Gln Glu Val Gly	Lys Ala Met Tyr	Ala Pro Pro Ile	Ser Gly Gln Ile
	435		440
Arg Cys Ser Ser	Asn Ile Thr Gly	Leu Leu Leu Thr	Arg Asp Gly Gly
	450		455
			460

Asn Asn Asn Asn Gly Ser Glu Ile Phe Arg Pro Gly Gly Gly Asp Met  
 465 470 475 480  
 Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile  
 485 490 495  
 Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln  
 500 505 510  
 Arg Glu Lys Arg Ala Val Gly Ile Gly Ala Leu Phe Leu Gly Phe Leu  
 515 520 525  
 Gly Ala Ala Gly Ser Thr Met Gly Ala Arg Ser Met Thr Leu Thr Val  
 530 535 540  
 Gln Ala Arg Gln Leu Leu Ser Gly Ile Val Gln Gln Gln Asn Asn Leu  
 545 550 555 560  
 Leu Arg Ala Ile Glu Ala Gln Gln His Leu Leu Gln Leu Thr Val Trp  
 565 570 575  
 Gly Ile Lys Gln Leu Gln Ala Arg Ile Leu Ala Val Glu Arg Tyr Leu  
 580 585 590  
 Lys Asp Gln Gln Leu Leu Gly Ile Trp Gly Cys Ser Gly Lys Leu Ile  
 595 600 605  
 Cys Thr Thr Ala Val Pro Trp Asn Ala Ser Trp Ser Asn Lys Ser Leu  
 610 615 620  
 Glu Gln Ile Trp Asn Asn Met Thr Trp Met Glu Trp Asp Arg Glu Ile  
 625 630 635 640  
 Asn Asn Tyr Thr Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn  
 645 650 655  
 Gln Gln Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala  
 660 665 670  
 Ser Leu Trp Asn Trp Phe Asn Ile Thr Asn Trp Leu Trp Tyr Ile Lys  
 675 680 685  
 Ile Phe Ile Met Ile Val Gly Gly Leu Val Gly Leu Arg Ile Val Phe  
 690 695 700  
 Ala Val Leu Ser Ile Val Asn Arg Val Arg Gln Gly Tyr Ser Pro Leu  
 705 710 715 720  
 Ser Phe Gln Thr His Leu Pro Thr Pro Arg Gly Pro Asp Arg Pro Glu  
 725 730 735  
 Gly Ile Glu Glu Glu Gly Gly Glu Arg Asp Arg Asp Arg Ser Ile Arg  
 740 745 750  
 Leu Val Asn Gly Ser Leu Ala Leu Ile Trp Asp Asp Leu Arg Ser Leu  
 755 760 765

Cys Leu Phe Ser Tyr His Arg Leu Arg Asp Leu Leu Leu Ile Val Thr  
 770 775 780  
 Arg Ile Val Glu Leu Leu Gly Arg Arg Gly Trp Glu Ala Leu Lys Tyr  
 785 790 795 800  
 Trp Trp Asn Leu Leu Gln Tyr Trp Ser Gln Glu Leu Lys Asn Ser Ala  
 805 810 815  
 Val Ser Leu Leu Asn Ala Thr Ala Ile Ala Val Ala Glu Gly Thr Asp  
 820 825 830  
 Arg Val Ile Glu Val Val Gln Gly Ala Cys Arg Ala Ile Arg His Ile  
 835 840 845  
 Pro Arg Arg Ile Arg Gln Gly Leu Glu Arg Ile Leu Leu Xaa  
 850 855 860

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 856 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Lys Val Lys Gly Thr Arg Arg Asn Tyr Gln His Leu Trp Arg Trp  
 1 5 10 15  
 Gly Thr Leu Leu Leu Gly Met Leu Met Ile Cys Ser Ala Thr Glu Lys  
 20 25 30  
 Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Thr  
 35 40 45  
 Thr Thr Leu Phe Cys Ala Ser Asp Ala Arg Ala Tyr Asp Thr Glu Val  
 50 55 60  
 His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro  
 65 70 75 80  
 Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met Trp Lys  
 85 90 95  
 Asn Asn Met Val Glu Gln Met Gln Glu Asp Ile Ile Ser Leu Trp Asp  
 100 105 110  
 Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu  
 115 120 125

Asn Cys Thr Asp Leu Gly Lys Ala Thr Asn Thr Asn Ser Ser Asn Trp  
 130 135 140  
 Lys Glu Glu Ile Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Thr  
 145 150 155 160  
 Thr Ser Ile Arg Asp Lys Ile Gln Lys Glu Asn Ala Leu Phe Arg Asn  
 165 170 175  
 Leu Asp Val Val Pro Ile Asp Asn Ala Ser Thr Thr Thr Asn Tyr Thr  
 180 185 190  
 Asn Tyr Arg Leu Ile His Cys Asn Arg Ser Val Ile Thr Gln Ala Cys  
 195 200 205  
 Pro Lys Val Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Thr Pro Ala  
 210 215 220  
 Gly Phe Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Lys Gly  
 225 230 235 240  
 Pro Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro  
 245 250 255  
 Ile Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu  
 260 265 270  
 Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile  
 275 280 285  
 Val Gln Leu Asn Glu Ser Val Ala Ile Asn Cys Thr Arg Pro Asn Asn  
 290 295 300  
 Asn Thr Arg Lys Ser Ile Tyr Ile Gly Pro Gly Arg Ala Phe His Thr  
 305 310 315 320  
 Thr Gly Arg Ile Ile Gly Asp Ile Arg Lys Ala His Cys Asn Ile Ser  
 325 330 335  
 Arg Ala Gln Trp Asn Asn Thr Leu Glu Gln Ile Val Lys Lys Leu Arg  
 340 345 350  
 Glu Gln Phe Gly Asn Asn Lys Thr Ile Val Phe Asn Gln Ser Ser Gly  
 355 360 365  
 Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Arg Gly Glu Phe  
 370 375 380  
 Phe Tyr Cys Asn Thr Thr Gln Leu Phe Asn Asn Thr Trp Arg Leu Asn  
 385 390 395 400  
 His Thr Glu Gly Thr Lys Gly Asn Asp Thr Ile Ile Leu Pro Cys Arg  
 405 410 415  
 Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr  
 420 425 430

Ala Pro Pro Ile Gly Gly Gln Ile Ser Cys Ser Ser Asn Ile Thr Gly  
 435 440 445  
 Leu Leu Leu Thr Arg Asp Gly Gly Thr Asn Val Thr Asn Asp Thr Glu  
 450 455 460  
 Val Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu  
 465 470 475 480  
 Leu Tyr Lys Tyr Lys Val Ile Lys Ile Glu Pro Leu Gly Ile Ala Pro  
 485 490 495  
 Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly  
 500 505 510  
 Ile Val Gly Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr  
 515 520 525  
 Met Gly Ala Val Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu  
 530 535 540  
 Ser Gly Ile Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala  
 545 550 555 560  
 Gln Gln His Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln  
 565 570 575  
 Ala Arg Val Leu Ala Val Glu Arg Tyr Leu Arg Asp Gln Gln Leu Leu  
 580 585 590  
 Gly Ile Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro  
 595 600 605  
 Trp Asn Ala Ser Trp Ser Asn Lys Ser Leu Glu Asp Ile Trp Asp Asn  
 610 615 620  
 Met Thr Trp Met Gln Trp Glu Arg Glu Ile Asp Asn Tyr Thr Asn Thr  
 625 630 635 640  
 Ile Tyr Thr Leu Leu Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu  
 645 650 655  
 Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe  
 660 665 670  
 Ser Ile Thr Asn Trp Leu Trp Tyr Ile Lys Ile Phe Ile Met Ile Val  
 675 680 685  
 Gly Gly Leu Val Gly Leu Arg Ile Val Phe Ala Val Leu Ser Ile Val  
 690 695 700  
 Asn Arg Val Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln Thr Arg Leu  
 705 710 715 720  
 Pro Val Pro Arg Gly Pro Asp Arg Pro Asp Gly Ile Glu Glu Glu Gly  
 725 730 735



Gly Glu Arg Asp Arg Asp Arg Ser Val Arg Leu Val Asp Gly Phe Leu  
740 745 750

Ala Leu Ile Trp Glu Asp Leu Arg Ser Leu Cys Leu Phe Ser Tyr Arg  
755 760 765

Arg Leu Arg Asp Leu Leu Leu Ile Ala Ala Arg Thr Val Glu Ile Leu  
770 775 780

Gly His Arg Gly Trp Glu Ala Leu Lys Tyr Trp Trp Ser Leu Leu Gln  
785 790 795 800

Tyr Trp Ile Gln Glu Leu Lys Asn Ser Ala Val Ser Trp Leu Asn Ala  
805 810 815

Thr Ala Ile Ala Val Thr Glu Gly Thr Asp Arg Val Ile Glu Val Ala  
820 825 830

Gln Arg Ala Tyr Arg Ala Ile Leu His Ile His Arg Arg Ile Arg Gln  
835 840 845

Gly Leu Glu Arg Leu Leu Leu Xaa  
850 855

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 854 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..854
- (D) OTHER INFORMATION: /note= "Xaa indicates residues

which are not specified in the consensus sequence."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Arg Xaa Xaa Glu Asn Xaa Xaa Xaa Xaa Xaa Xaa Xaa Trp Lys  
1 5 10 15

Trp Gly Ile Met Leu Leu Gly Met Leu Met Xaa Ser Ala Xaa Glu Xaa  
20 25 30

Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Xaa Lys Glu Thr Thr Thr  
35 40 45

Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Xaa Xaa Glu Xaa His Asn  
50 55 60

Xaa Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro Gln Glu  
 65 70 75 80  
 Ile Xaa Leu Xaa Asn Val Thr Glu Xaa Phe Asn Met Trp Lys Asn Xaa  
 85 90 95  
 Met Val Glu Gln Met His Xaa Asp Ile Ile Ser Leu Trp Asp Xaa Ser  
 100 105 110  
 Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu Asn Cys  
 115 120 125  
 Thr Asp Leu Lys Asn Glu Thr Xaa Xaa Asn Ser Ser Xaa Xaa Asn Xaa  
 130 135 140  
 Xaa Xaa Xaa Glu Ile Met Gly Xaa Glu Xaa Xaa Lys Asn Cys Ser Phe  
 145 150 155 160  
 Asn Val Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Tyr Xaa Leu  
 165 170 175  
 Phe Xaa Xaa Xaa Xaa Ile Val Xaa Xaa Xaa Asn Xaa Xaa Asn Ser Thr  
 180 185 190  
 Xaa Xaa Xaa Leu Xaa Xaa Cys Xaa Thr Ser Xaa Xaa Thr Gln Ala Cys  
 195 200 205  
 Pro Lys Xaa Ser Phe Glu Pro Ile Pro Ile His Xaa Cys Ala Pro Ala  
 210 215 220  
 Gly Phe Ala Ile Leu Lys Cys Xaa Asp Lys Lys Phe Asn Gly Thr Gly  
 225 230 235 240  
 Pro Cys Xaa Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro  
 245 250 255  
 Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Xaa Ala Glu Glu Glu  
 260 265 270  
 Xaa Xaa Xaa Arg Ser Xaa Asn Xaa Xaa Xaa Asn Xaa Lys Thr Ile Ile  
 275 280 285  
 Val Gln Leu Asn Xaa Ser Xaa Xaa Ile Xaa Cys Thr Arg Pro Xaa Xaa  
 290 295 300  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ile Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 305 310 315 320  
 Xaa Thr Gly Lys Xaa Xaa Lys Xaa Gly Xaa Xaa Xaa Gln Ala His Cys  
 325 330 335  
 Xaa Xaa Xaa Arg Xaa Xaa Trp Xaa Xaa Ala Leu Xaa Gln Xaa Ala Xaa  
 340 345 350  
 Lys Leu Xaa Xaa Xaa Xaa Asn Lys Thr Xaa Ile Xaa Phe Xaa Xaa Ser  
 355 360 365

Ser Gly Gly Asp Xaa Glu Ile Xaa Xaa His Xaa Xaa Asn Cys Gly Gly  
 370 375 380  
 Xaa Xaa Phe Tyr Cys Asn Thr Xaa Xaa Leu Phe Asn Ser Thr Trp Asn  
 385 390 395 400  
 Xaa Thr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Thr Leu Pro Cys  
 405 410 415  
 Arg Ile Xaa Gln Xaa Val Asn Xaa Trp Gln Xaa Val Gly Lys Ala Met  
 420 425 430  
 Tyr Ala Pro Pro Xaa Xaa Gly Xaa Ile Xaa Cys Ser Asn Ile Thr Gly  
 435 440 445  
 Leu Leu Leu Thr Arg Asp Gly Gly His Asn Xaa Asn Asn Xaa Xaa Xaa  
 450 455 460  
 Glu Thr Xaa Arg Pro Gly Xaa Gly Asp Met Arg Asp Asn Trp Arg Ser  
 465 470 475 480  
 Glu Leu Tyr Lys Tyr Lys Val Xaa Lys Ile Glu Pro Xaa Gly Val Ala  
 485 490 495  
 Pro Thr Lys Ala Xaa Arg Arg Val Val Xaa Arg Glu Lys Arg Ala Xaa  
 500 505 510  
 Met Gly Ala Xaa Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met  
 515 520 525  
 Gly Ala Ala Ser Xaa Leu Thr Val Gln Ala Arg Gln Leu Xaa Ser Gly  
 530 535 540  
 Ile Val Xaa Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln  
 545 550 555 560  
 His Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg  
 565 570 575  
 Val Leu Ala Val Glu Arg Tyr Leu Xaa Asp Gln Gln Leu Leu Gly Ile  
 580 585 590  
 Trp Gly Cys Ser Gly Xaa Xaa Ile Cys Thr Thr Xaa Val Pro Trp Asn  
 595 600 605  
 Xaa Ser Trp Ser Asn Xaa Ser Leu Asp Xaa Ile Trp Xaa Asn Met Thr  
 610 615 620  
 Trp Xaa Glu Trp Xaa Arg Glu Ile Xaa Asn Tyr Thr Xaa Leu Ile Xaa  
 625 630 635 640  
 Xaa Leu Ile Glu Glu Ser Gln Xaa Gln Gln Glu Lys Asn Glu Xaa Glu  
 645 650 655  
 Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp Xaa Trp Phe Xaa Ile  
 660 665 670

Xaa Xaa Trp Leu Trp Tyr Ile Lys Xaa Phe Ile Met Ile Val Xaa Gly  
675 680 685

Leu Xaa Gly Leu Arg Ile Val Phe Ala Val Leu Ser Xaa Val Asn Arg  
690 695 700

Val Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln Thr Xaa Xaa Pro Xaa  
705 710 715 720

Pro Arg Gly Pro Asp Arg Pro Xaa Xaa Ile Glu Xaa Glu Gly Gly Glu  
725 730 735

Arg Xaa Arg Asp Arg Ser Xaa Arg Leu Val Xaa Gly Xaa Xaa Ala Leu  
740 745 750

Xaa Trp Xaa Asp Leu Arg Asn Leu Cys Leu Phe Ser Tyr His Arg Leu  
755 760 765

Arg Asp Xaa Xaa Leu Ile Xaa Xaa Arg Ile Val Glu Leu Leu Gly Arg  
770 775 780

Arg Gly Trp Glu Ala Leu Lys Tyr Leu Trp Xaa Leu Leu Gln Tyr Trp  
785 790 795 800

Ser Gln Glu Leu Xaa Asn Ser Ala Xaa Xaa Leu Xaa Xaa Thr Xaa Ala  
805 810 815

Ile Xaa Val Ala Glu Xaa Thr Asp Arg Xaa Ile Glu Val Val Gln Arg  
820 825 830

Xaa Cys Arg Ala Ile Leu Xaa Xaa Pro Arg Arg Ile Arg Gln Gly Leu  
835 840 845

Glu Arg Xaa Leu Leu Xaa  
850

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 866 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..866
- (D) OTHER INFORMATION: /note= "Xaa residues are not specified in this consensus sequence."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

Met Arg Val Lys Glu Xaa Xaa Gln His Leu Trp Arg Trp Gly Trp Lys
1           5           10           15

Trp Gly Xaa Met Leu Leu Gly Xaa Leu Met Ile Cys Ser Ala Thr Glu
20           25           30

Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Xaa
35           40           45

Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Xaa Xaa Glu
50           55           60

Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn
65           70           75           80

Pro Gln Glu Xaa Val Leu Xaa Asn Val Thr Glu Xaa Phe Asn Met Trp
85           90           95

Lys Asn Xaa Met Val Glu Gln Met His Xaa Asp Ile Ile Ser Leu Trp
100          105          110

Asp Xaa Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Xaa
115          120          125

Leu Xaa Cys Thr Asp Leu Xaa Asn Xaa Thr Xaa Thr Asn Ser Ser Xaa
130          135          140

Xaa Asn Ser Xaa Ser Gly Glu Xaa Met Met Xaa Xaa Xaa Glu Ile Lys
145          150          155          160

Asn Cys Ser Phe Asn Xaa Ser Thr Xaa Xaa Xaa Gly Lys Val Gln Lys
165          170          175

Glu Tyr Xaa Xaa Phe Tyr Xaa Leu Asp Ile Val Ser Ile Xaa Xaa Xaa
180          185          190

Asn Xaa Xaa Thr Ser Xaa Xaa Leu Thr Ser Cys Asn Thr Ser Val Xaa
195          200          205

Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro Ile His Tyr
210          215          220

Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Xaa Lys Xaa Phe
225          230          235          240

Asn Gly Thr Gly Pro Cys Xaa Asn Val Ser Thr Val Gln Cys Thr His
245          250          255

Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Xaa
260          265          270

Ala Glu Glu Glu Val Val Xaa Arg Ser Ala Asn Phe Xaa Asp Asn Ala
275          280          285

Lys Thr Ile Ile Val Gln Leu Asn Xaa Ser Val Glu Ile Xaa Cys Thr
290          295          300

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Arg Pro Asn Xaa Asn Xaa Xaa Lys Xaa Ile Arg Ile Xaa Arg Gly Xaa  
 305 310 315 320  
 Gly Arg Xaa Phe Val Thr Xaa Xaa Lys Xaa Gly Xaa Xaa Xaa Gln Ala  
 325 330 335  
 His Cys Xaa Xaa Xaa Arg Xaa Lys Trp Xaa Xaa Xaa Leu Lys Gln Ile  
 340 345 350  
 Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn Xaa Xaa Xaa Ile Ile Phe  
 355 360 365  
 Xaa Xaa Ser Ser Gly Gly Asp Xaa Glu Ile Xaa Xaa His Ser Phe Asn  
 370 375 380  
 Cys Gly Gly Glu Xaa Phe Tyr Cys Asn Xaa Thr Xaa Leu Phe Asn Ser  
 385 390 395 400  
 Thr Trp Phe Asn Xaa Thr Trp Ser Thr Glu Gly Ser Asn Asn Xaa Xaa  
 405 410 415  
 Gly Ser Xaa Xaa Ile Thr Leu Pro Cys Arg Ile Xaa Gln Phe Xaa Asn  
 420 425 430  
 Met Trp Gln Xaa Val Gly Lys Ala Met Tyr Ala Pro Pro Xaa Xaa Gly  
 435 440 445  
 Gln Ile Arg Cys Xaa Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp  
 450 455 460  
 Gly Gly His Asn Asp Asn Asn Xaa Xaa Xaa Glu Xaa Phe Arg Pro Gly  
 465 470 475 480  
 Xaa Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys  
 485 490 495  
 Val Xaa Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys Arg  
 500 505 510  
 Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Met Xaa Gly Ala Xaa  
 515 520 525  
 Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala Xaa Ser  
 530 535 540  
 Xaa Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile Val Gln  
 545 550 555 560  
 Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His Leu Leu  
 565 570 575  
 Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Xaa Leu Ala  
 580 585 590  
 Val Glu Arg Tyr Leu Lys Asp Gln Gln Leu Leu Gly Ile Trp Gly Cys  
 595 600 605

Ser Gly Lys Leu Ile Cys Thr Thr Xaa Val Pro Trp Asn Ala Ser Trp  
 610 615 620  
 Ser Asn Lys Ser Leu Xaa Gln Ile Trp Asn Asn Met Thr Trp Xaa Glu  
 625 630 635 640  
 Trp Asp Arg Glu Ile Xaa Asn Thr Xaa Leu Ile His Xaa Leu Ile Glu  
 645 650 655  
 Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu  
 660 665 670  
 Asp Lys Trp Ala Ser Leu Trp Xaa Trp Phe Xaa Ile Xaa Asn Trp Leu  
 675 680 685  
 Trp Tyr Ile Lys Ile Phe Ile Met Ile Val Xaa Gly Leu Val Gly Leu  
 690 695 700  
 Arg Ile Val Phe Ala Val Leu Ser Ile Val Asn Arg Val Arg Gln Gly  
 705 710 715 720  
 Tyr Ser Pro Leu Ser Phe Gln Thr His Xaa Pro Xaa Pro Arg Gly Pro  
 725 730 735  
 Asp Arg Pro Xaa Gly Ile Glu Xaa Glu Gly Gly Glu Arg Asp Arg Asp  
 740 745 750  
 Arg Ser Xaa Arg Leu Val Xaa Gly Xaa Leu Ala Leu Xaa Trp Xaa Asp  
 755 760 765  
 Leu Arg Xaa Leu Cys Leu Phe Ser Tyr His Arg Leu Arg Asp Leu Leu  
 770 775 780  
 Leu Ile Val Thr Arg Ile Val Glu Leu Leu Gly Arg Arg Gly Trp Glu  
 785 790 795 800  
 Ala Leu Lys Tyr Xaa Trp Xaa Leu Leu Gln Tyr Trp Ser Gln Glu Leu  
 805 810 815  
 Lys Asn Ser Ala Val Xaa Leu Xaa Asn Xaa Thr Ala Ile Xaa Val Ala  
 820 825 830  
 Glu Gly Thr Asp Arg Xaa Ile Glu Val Val Gln Xaa Xaa Cys Arg Ala  
 835 840 845  
 Ile Xaa His Ile Pro Arg Arg Ile Arg Gln Gly Leu Glu Arg Xaa Leu  
 850 855 860  
 Leu Xaa  
 865

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 836 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 1..836

(D) OTHER INFORMATION: /note= "Xaa residues are not specified in this consensus sequence."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Met Val Lys Glu Trp Trp Gly Leu Leu Gly Met Leu Met Ile Cys Ser
 1             5             10             15

Ala Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp
      20             25             30

Lys Glu Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Ala Tyr Glu Val
      35             40             45

His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro
      50             55             60

Gln Glu Val Leu Asn Val Thr Glu Phe Asn Met Trp Lys Asn Met Val
      65             70             75             80

Glu Gln Met Asp Ile Ile Ser Leu Trp Asp Ser Leu Lys Pro Cys Val
      85             90             95

Lys Leu Thr Pro Leu Cys Val Thr Leu Asn Cys Thr Asp Leu Xaa Xaa
      100            105            110

Xaa Thr Xaa Thr Asn Ser Ser Asp Ala Asn Xaa Xaa Xaa Xaa Xaa
      115            120            125

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
      130            135            140

Xaa Xaa Xaa Lys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
      145            150            155            160

Ile Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Thr Xaa Xaa Xaa Leu Xaa
      165            170            175

Xaa Cys Asn Xaa Ser Val Xaa Thr Gln Ala Cys Pro Lys Val Ser Phe
      180            185            190

Glu Pro Ile Pro Ile His Tyr Cys Xaa Pro Ala Gly Phe Ala Ile Leu
      195            200            205

Lys Cys Asn Xaa Lys Xaa Phe Asn Gly Xaa Gly Pro Cys Xaa Asn Val
      210            215            220

```



```

Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Xaa Val Ser Thr Gln
225                230                235                240

Leu Leu Leu Asn Gly Ser Xaa Ala Glu Glu Glu Val Val Xaa Arg Ser
245                250                255

Xaa Asn Phe Xaa Xaa Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Xaa
260                265                270

Ser Val Xaa Ile Xaa Cys Thr Arg Pro Asn Xaa Asn Xaa Xaa Lys Xaa
275                280                285

Ile Xaa Ile Xaa Xaa Gly Tyr Xaa Xaa Xaa Xaa Xaa Thr Xaa Arg Xaa
290                295                300

Xaa Gly Asp Xaa Xaa Xaa Ala His Cys Xaa Xaa Xaa Arg Xaa Xaa Trp
305                310                315                320

Xaa Asn Xaa Leu Xaa Gln Ile Xaa Xaa Lys Leu Arg Glu Gln Phe Gly
325                330                335

Asn Xaa Xaa Xaa Ile Xaa Phe Asn Xaa Ser Ser Gly Gly Asp Xaa Glu
340                345                350

Ile Xaa Met His Ser Phe Asn Cys Xaa Gly Glu Xaa Phe Tyr Cys Asn
355                360                365

Thr Thr Xaa Leu Phe Asn Xaa Thr Trp Arg Xaa Xaa Xaa Thr Glu Xaa
370                375                380

Xaa Xaa Xaa Xaa Xaa Xaa Ile Xaa Leu Pro Cys Arg Ile Xaa Gln Xaa
385                390                395                400

Xaa Asn Met Trp Gln Xaa Val Gly Lys Ala Met Tyr Ala Pro Pro Xaa
405                410                415

Xaa Gly Gln Ile Xaa Cys Xaa Ser Asn Ile Thr Gly Leu Leu Leu Thr
420                425                430

Arg Asp Gly Gly Xaa Asn Xaa Xaa Asn Thr Xaa Xaa Glu Xaa Phe Arg
435                440                445

Pro Gly Xaa Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys
450                455                460

Tyr Lys Val Ile Lys Ile Glu Pro Leu Gly Xaa Ala Pro Thr Lys Ala
465                470                475                480

Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Xaa Val Gly
485                490                495

Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala
500                505                510

Xaa Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile Val
515                520                525

```

Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His Leu  
 530 535 540

Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val Leu  
 545 550 555 560

Ala Val Glu Arg Tyr Leu Xaa Asp Gln Gln Leu Leu Gly Ile Trp Gly  
 565 570 575

Cys Ser Gly Lys Leu Ile Cys Thr Thr Xaa Val Pro Trp Asn Ala Ser  
 580 585 590

Trp Ser Asn Lys Ser Leu Xaa Xaa Ile Trp Xaa Asn Met Thr Trp Xaa  
 595 600 605

Xaa Trp Xaa Arg Glu Ile Xaa Asn Tyr Thr Asn Xaa Ile Xaa Xaa Leu  
 610 615 620

Xaa Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Gln Glu Leu Leu  
 625 630 635 640

Glu Leu Asp Lys Trp Ala Ser Leu Trp Xaa Trp Phe Xaa Ile Xaa Asn  
 645 650 655

Trp Leu Trp Tyr Ile Lys Ile Phe Ile Met Ile Val Xaa Gly Leu Val  
 660 665 670

Gly Leu Arg Ile Val Phe Ala Val Leu Ser Ile Val Asn Arg Val Arg  
 675 680 685

Gln Gly Tyr Ser Pro Leu Ser Phe Gln Thr Xaa Xaa Pro Xaa Pro Arg  
 690 695 700

Gly Pro Asp Arg Pro Asp Gly Ile Glu Xaa Glu Gly Gly Glu Arg Asp  
 705 710 715 720

Arg Asp Arg Ser Val Arg Leu Val Asp Gly Phe Leu Ala Leu Xaa Trp  
 725 730 735

Glu Asp Leu Arg Xaa Leu Cys Leu Phe Ser Tyr Xaa Arg Leu Arg Asp  
 740 745 750

Leu Leu Leu Ile Xaa Xaa Arg Xaa Val Glu Xaa Leu Gly Xaa Arg Gly  
 755 760 765

Trp Glu Ala Leu Lys Tyr Xaa Trp Ser Leu Leu Gln Tyr Trp Xaa Gln  
 770 775 780

Glu Leu Lys Asn Ser Ala Val Xaa Xaa Xaa Asn Xaa Thr Ala Ile Xaa  
 785 790 795 800

Val Xaa Glu Gly Thr Asp Arg Xaa Ile Glu Val Xaa Gln Arg Xaa Xaa  
 805 810 815

Arg Ala Ile Leu His Ile Xaa Arg Arg Ile Arg Gln Gly Leu Glu Arg  
 820 825 830

Xaa Leu Leu Xaa  
835

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "Oligonucleotide."

## (iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCCTTCGAAG AGGATATAAT CAGTTTATGG GATCAAAGC

39

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "Oligonucleotide."

## (iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCCTTCGAAC TCTTCTTCTG CTAGACTGCC ATT

33

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9193 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: not relevant

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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PCT/US98/12990

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gaaagaatatagcacttttttatgcacttgatatagtatcaataaagaatgaaaaataatagtaccagccat  
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aatcgaaggagaaggtggagagagagacagagacagatccgtgcgattagtggtggattcttagcactt  
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tatccttgatctgtggatctaccacacacaaggctacttccctgattggcagaactacacaccaggcca  
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gtctctctgggttagaccagatctgagcctgggagctctctggctagctaggaacccactgcttaagcct  
caataaagcttgcccttgagtgttca

FOR SEQ ID NO:13 THROUGH SEQ ID NO:17, PLEASE SEE TABLE 5.

(2) INFORMATION FOR SEQ ID NO:18

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc = "Oligonucleotide."
- (iii) HYPOTHETICAL: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18

AAATCTCTAG CAGTGGCGCC CGAACAG

27

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc = "Oligonucleotide."
- (iii) HYPOTHETICAL: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCACTCAAGG CAAGCTTTAT TGAGGCT

27

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 31 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc = "Oligonucleotide."
- (iii) HYPOTHETICAL: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CACACACAAG GCTACTTCCC TGATTGGCAG A

31

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 33 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATGGAACAAG CCCCAGAAGA CCAAGGGCCA CAG

33

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 31 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGTCTGAGGG ATCTCTAGTT ACCAGAGTCA C

31

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2404 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met	Glu	Thr	Ala	Arg	Gly	Val	Ala	Leu	Leu	Tyr	Ser	Gly	Leu	Ala	Ser
1				5				10					15		
Asn	Thr	Tyr	Arg	Gly	Leu	Asn	His	Ile	Ser	Leu	Glu	Thr	Arg	Pro	Ala
			20				25						30		
Arg	Gly	Thr	Arg	Pro	Gly	Leu	Tyr	Thr	Arg	Pro	Leu	Tyr	Ser	Thr	Arg
			35				40						45		
Pro	Gly	Leu	Tyr	Ile	Leu	Glu	Met	Glu	Thr	Leu	Glu	Leu	Glu	Gly	Leu
			50				55						60		

Tyr Met Glu Thr Leu Glu Met Glu Thr Ile Leu Glu Cys Tyr Ser Ser  
 65 70 75 80  
 Glu Arg Ala Leu Ala Thr His Arg Gly Leu Leu Tyr Ser Leu Glu Thr  
 85 90 95  
 Arg Pro Val Ala Leu Thr His Arg Val Ala Leu Thr Tyr Arg Thr Tyr  
 100 105 110  
 Arg Gly Leu Tyr Val Ala Leu Pro Arg Val Ala Leu Thr Arg Pro Leu  
 115 120 125  
 Tyr Ser Gly Leu Ala Leu Ala Thr His Arg Thr His Arg Thr His Arg  
 130 135 140  
 Leu Glu Pro His Glu Cys Tyr Ser Ala Leu Ala Ser Glu Arg Ala Ser  
 145 150 155 160  
 Pro Ala Leu Ala Leu Tyr Ser Ala Leu Ala Thr Tyr Arg Gly Leu Gly  
 165 170 175  
 Leu Gly Leu Val Ala Leu His Ile Ser Ala Ser Asn Val Ala Leu Thr  
 180 185 190  
 Arg Pro Ala Leu Ala Thr His Arg His Ile Ser Ala Leu Ala Cys Tyr  
 195 200 205  
 Ser Val Ala Leu Pro Arg Thr His Arg Ala Ser Pro Pro Arg Ala Ser  
 210 215 220  
 Asn Pro Arg Gly Leu Asn Gly Leu Ile Leu Glu Val Ala Leu Leu Glu  
 225 230 235 240  
 Ala Leu Ala Ala Ser Asn Val Ala Leu Thr His Arg Gly Leu Ala Ser  
 245 250 255  
 Pro Pro His Glu Ala Ser Asn Met Glu Thr Thr Arg Pro Leu Tyr Ser  
 260 265 270  
 Ala Ser Asn Gly Leu Met Glu Thr Val Ala Leu Gly Leu Gly Leu Asn  
 275 280 285  
 Met Glu Thr His Ile Ser Thr His Arg Ala Ser Pro Ile Leu Glu Ile  
 290 295 300  
 Leu Glu Ser Glu Arg Leu Glu Thr Arg Pro Ala Ser Pro Gly Leu Ser  
 305 310 315 320  
 Glu Arg Leu Glu Leu Tyr Ser Pro Arg Cys Tyr Ser Val Ala Leu Leu  
 325 330 335  
 Tyr Ser Leu Glu Thr His Arg Pro Arg Leu Glu Cys Tyr Ser Val Ala  
 340 345 350  
 Leu Thr His Arg Leu Glu Ala Ser Asn Cys Tyr Ser Thr His Arg Ala  
 355 360 365

Ser Pro Leu Glu Leu Tyr Ser Ala Ser Asn Gly Leu Thr His Arg Leu  
 370 375 380  
 Tyr Ser Thr His Arg Ala Ser Asn Ser Glu Arg Ser Glu Arg Ala Ser  
 385 390 395 400  
 Pro Ala Leu Ala Ala Ser Asn Ser Glu Arg Ala Ser Asn Ser Glu Arg  
 405 410 415  
 Gly Leu Tyr Gly Leu Ile Leu Glu Met Glu Thr Gly Leu Tyr Ala Ser  
 420 425 430  
 Asn Gly Leu Gly Leu Ile Leu Glu Leu Tyr Ser Ala Ser Asn Cys Tyr  
 435 440 445  
 Ser Ser Glu Arg Pro His Glu Ala Ser Asn Val Ala Leu Ser Glu Arg  
 450 455 460  
 Thr His Arg Gly Leu Tyr Ala Leu Ala Pro Arg Gly Leu Tyr Leu Tyr  
 465 470 475 480  
 Ser Val Ala Leu Gly Leu Asn Leu Tyr Ser Gly Leu Thr Tyr Arg Ser  
 485 490 495  
 Glu Arg Leu Glu Pro His Glu Thr Tyr Arg Ala Leu Ala Leu Glu Ala  
 500 505 510  
 Ser Pro Ile Leu Glu Val Ala Leu Ser Glu Arg Ile Leu Glu Leu Tyr  
 515 520 525  
 Ser Ala Ser Asn Gly Leu Ala Ser Asn Ala Ser Asn Ser Glu Arg Thr  
 530 535 540  
 His Arg Ser Glu Arg His Ile Ser Met Glu Thr Leu Glu Thr His Arg  
 545 550 555 560  
 Ser Glu Arg Cys Tyr Ser Ala Ser Asn Thr His Arg Ser Glu Arg Val  
 565 570 575  
 Ala Leu Ser Glu Arg Thr His Arg Gly Leu Asn Ala Leu Ala Cys Tyr  
 580 585 590  
 Ser Pro Arg Leu Tyr Ser Val Ala Leu Ser Glu Arg Pro His Glu Gly  
 595 600 605  
 Leu Pro Arg Ile Leu Glu Pro Arg Ile Leu Glu His Ile Ser Thr Tyr  
 610 615 620  
 Arg Cys Tyr Ser Ala Leu Ala Pro Arg Ala Leu Ala Gly Leu Tyr Pro  
 625 630 635 640  
 His Glu Ala Leu Ala Ile Leu Glu Leu Glu Leu Tyr Ser Cys Tyr Ser  
 645 650 655  
 Ala Ser Asn Ala Ser Pro Leu Tyr Ser Leu Tyr Ser Pro His Glu Ala  
 660 665 670



Ser Asn Gly Leu Tyr Thr His Arg Gly Leu Tyr Pro Arg Cys Tyr Ser  
 675 680 685  
 Ala Ser Asn Ala Ser Asn Val Ala Leu Ser Glu Arg Thr His Arg Val  
 690 695 700  
 Ala Leu Gly Leu Asn Cys Tyr Ser Thr His Arg His Ile Ser Gly Leu  
 705 710 715 720  
 Tyr Ile Leu Glu Ala Arg Gly Pro Arg Val Ala Leu Val Ala Leu Ser  
 725 730 735  
 Glu Arg Thr His Arg Gly Leu Asn Leu Glu Leu Glu Leu Glu Ala Ser  
 740 745 750  
 Asn Gly Leu Tyr Ser Glu Arg Leu Glu Ala Leu Ala Gly Leu Gly Leu  
 755 760 765  
 Gly Leu Val Ala Leu Val Ala Leu Leu Glu Ala Arg Gly Ser Glu Arg  
 770 775 780  
 Ala Leu Ala Ala Ser Asn Pro His Glu Ser Glu Arg Ala Ser Pro Ala  
 785 790 795 800  
 Ser Asn Ala Leu Ala Leu Tyr Ser Thr His Arg Ile Leu Glu Ile Leu  
 805 810 815  
 Glu Val Ala Leu Gly Leu Asn Leu Glu Ala Ser Asn His Ile Ser Ser  
 820 825 830  
 Glu Arg Val Ala Leu Gly Leu Ile Leu Glu Thr His Arg Cys Tyr Ser  
 835 840 845  
 Thr His Arg Ala Arg Gly Pro Arg Ala Ser Asn Thr Tyr Arg Ala Ser  
 850 855 860  
 Asn Gly Leu Thr His Arg Leu Tyr Ser Leu Tyr Ser Ile Leu Glu Ala  
 865 870 875 880  
 Arg Gly Ile Leu Glu His Ile Ser Ala Arg Gly Gly Leu Tyr Thr Tyr  
 885 890 895  
 Arg Gly Leu Tyr Ala Arg Gly Ser Glu Arg Pro His Glu Val Ala Leu  
 900 905 910  
 Thr His Arg Val Ala Leu Ala Arg Gly Leu Tyr Ser Leu Glu Gly Leu  
 915 920 925  
 Tyr Ala Ser Pro Ala Arg Gly Leu Tyr Ser Gly Leu Asn Ala Leu Ala  
 930 935 940  
 His Ile Ser Cys Tyr Ser Thr His Arg Met Glu Thr Ala Ser Asn Ala  
 945 950 955 960  
 Arg Gly Thr His Arg Leu Tyr Ser Thr Arg Pro Ala Ser Pro Ala Ser  
 965 970 975

Asn Ala Leu Ala Leu Glu Leu Tyr Ser Gly Leu Asn Ile Leu Glu Ala  
 980 985 990  
 Leu Ala Ser Glu Arg Leu Tyr Ser Leu Glu Ala Arg Gly Gly Leu Gly  
 995 1000 1005  
 Leu Asn Pro His Glu Ala Ser Asn Leu Tyr Ser Thr His Arg Ala Leu  
 1010 1015 1020  
 Ala Ile Leu Glu Ile Leu Glu Pro His Glu Ala Ser Asn Ala Arg Gly  
 1025 1030 1035 1040  
 Ser Glu Arg Ser Glu Arg Gly Leu Tyr Gly Leu Tyr Ala Ser Pro Leu  
 1045 1050 1055  
 Glu Gly Leu Ile Leu Glu Gly Leu Met Glu Thr His Ile Ser Ser Glu  
 1060 1065 1070  
 Arg Pro His Glu Ala Ser Asn Cys Tyr Ser Gly Leu Tyr Gly Leu Tyr  
 1075 1080 1085  
 Gly Leu Leu Glu Pro His Glu Thr Tyr Arg Cys Tyr Ser Ala Ser Asn  
 1090 1095 1100  
 Thr His Arg Thr His Arg Leu Tyr Ser Leu Glu Pro His Glu Ala Ser  
 1105 1110 1115 1120  
 Asn Ser Glu Arg Thr His Arg Thr Arg Pro Ala Ser Asn Gly Leu Thr  
 1125 1130 1135  
 His Arg Thr His Arg Gly Leu Ser Glu Arg Ala Ser Asn Gly Leu Tyr  
 1140 1145 1150  
 Leu Tyr Ser Gly Leu Tyr Gly Leu Ala Ser Asn Ile Leu Glu Thr His  
 1155 1160 1165  
 Arg Leu Glu Pro Arg Cys Tyr Ser Ala Arg Gly Ile Leu Glu Ala Arg  
 1170 1175 1180  
 Gly Gly Leu Asn Pro His Glu Val Ala Leu Ala Ser Asn Met Glu Thr  
 1185 1190 1195 1200  
 Thr Arg Pro Gly Leu Asn Leu Tyr Ser Val Ala Leu Gly Leu Tyr Leu  
 1205 1210 1215  
 Tyr Ser Ala Leu Ala Met Glu Thr Thr Tyr Arg Ala Leu Ala Pro Arg  
 1220 1225 1230  
 Pro Arg Ser Glu Arg Ala Ser Pro Gly Leu Tyr Gly Leu Asn Ile Leu  
 1235 1240 1245  
 Glu Ala Arg Gly Cys Tyr Ser Thr His Arg Ser Glu Arg Ala Ser Asn  
 1250 1255 1260  
 Ile Leu Glu Thr His Arg Gly Leu Tyr Leu Glu Leu Glu Leu Glu Thr  
 1265 1270 1275 1280

His Arg Ala Arg Gly Ala Ser Pro Gly Leu Tyr Gly Leu Tyr Gly Leu  
 1285 1290 1295  
 Tyr Pro Arg Ser Glu Arg Ala Ser Pro Ala Ser Asn Leu Tyr Ser Ala  
 1300 1305 1310  
 Ser Pro Leu Tyr Ser Gly Leu Thr His Arg Pro His Glu Ala Arg Gly  
 1315 1320 1325  
 Pro Arg Gly Leu Tyr Gly Leu Tyr Gly Leu Tyr Ala Ser Pro Met Glu  
 1330 1335 1340  
 Thr Ala Arg Gly Ala Ser Pro Ala Ser Asn Thr Arg Pro Ala Arg Gly  
 1345 1350 1355 1360  
 Ser Glu Arg Gly Leu Leu Glu Thr Tyr Arg Leu Tyr Ser Thr Tyr Arg  
 1365 1370 1375  
 Leu Tyr Ser Val Ala Leu Ile Leu Glu Leu Tyr Ser Ile Leu Glu Gly  
 1380 1385 1390  
 Leu Pro Arg Leu Glu Gly Leu Tyr Val Ala Leu Ala Leu Ala Pro Arg  
 1395 1400 1405  
 Thr His Arg Leu Tyr Ser Ala Leu Ala Leu Tyr Ser Ala Arg Gly Ala  
 1410 1415 1420  
 Arg Gly Val Ala Leu Val Ala Leu Gly Leu Asn Ala Arg Gly Gly Leu  
 1425 1430 1435 1440  
 Leu Tyr Ser Ala Arg Gly Ala Leu Ala Val Ala Leu Gly Leu Tyr Met  
 1445 1450 1455  
 Glu Thr Val Ala Leu Gly Leu Tyr Ala Leu Ala Met Glu Thr Pro His  
 1460 1465 1470  
 Glu Leu Glu Gly Leu Tyr Pro His Glu Leu Glu Gly Leu Tyr Ala Leu  
 1475 1480 1485  
 Ala Ala Leu Ala Gly Leu Tyr Ser Glu Arg Thr His Arg Met Glu Thr  
 1490 1495 1500  
 Gly Leu Tyr Ala Leu Ala Ala Leu Ala Ser Glu Arg Leu Glu Thr His  
 1505 1510 1515 1520  
 Arg Leu Glu Thr His Arg Val Ala Leu Gly Leu Asn Ala Leu Ala Ala  
 1525 1530 1535  
 Arg Gly Gly Leu Asn Leu Glu Leu Glu Ser Glu Arg Gly Leu Tyr Ile  
 1540 1545 1550  
 Leu Glu Val Ala Leu Gly Leu Asn Gly Leu Asn Gly Leu Asn Ala Ser  
 1555 1560 1565  
 Asn Ala Ser Asn Leu Glu Leu Glu Ala Arg Gly Ala Leu Ala Ile Leu  
 1570 1575 1580

Glu Gly Leu Ala Leu Ala Gly Leu Asn Gly Leu Asn His Ile Ser Leu  
 1585 1590 1595 1600  
 Glu Leu Glu Gly Leu Asn Leu Glu Thr His Arg Val Ala Leu Thr Arg  
 1605 1610 1615  
 Pro Gly Leu Tyr Ile Leu Glu Leu Tyr Ser Gly Leu Asn Leu Glu Gly  
 1620 1625 1630  
 Leu Asn Ala Leu Ala Ala Arg Gly Val Ala Leu Leu Glu Ala Leu Ala  
 1635 1640 1645  
 Val Ala Leu Gly Leu Ala Arg Gly Thr Tyr Arg Leu Glu Leu Tyr Ser  
 1650 1655 1660  
 Ala Ser Pro Gly Leu Asn Gly Leu Asn Leu Glu Leu Glu Gly Leu Tyr  
 1665 1670 1675 1680  
 Ile Leu Glu Thr Arg Pro Gly Leu Tyr Cys Tyr Ser Ser Glu Arg Gly  
 1685 1690 1695  
 Leu Tyr Leu Tyr Ser Leu Glu Ile Leu Glu Cys Tyr Ser Thr His Arg  
 1700 1705 1710  
 Thr His Arg Thr His Arg Val Ala Leu Pro Arg Thr Arg Pro Ala Ser  
 1715 1720 1725  
 Asn Ala Leu Ala Ser Glu Arg Thr Arg Pro Ser Glu Arg Ala Ser Asn  
 1730 1735 1740  
 Leu Tyr Ser Ser Glu Arg Leu Glu Ala Ser Pro Gly Leu Asn Ile Leu  
 1745 1750 1755 1760  
 Glu Thr Arg Pro Ala Ser Asn Ala Ser Asn Met Glu Thr Thr His Arg  
 1765 1770 1775  
 Thr Arg Pro Leu Glu Gly Leu Thr Arg Pro Ala Ser Pro Ala Arg Gly  
 1780 1785 1790  
 Gly Leu Ile Leu Glu Ala Leu Ala Ala Ser Asn Thr Tyr Arg Thr His  
 1795 1800 1805  
 Arg Ala Ser Asn Leu Glu Ile Leu Glu His Ile Ser His Ile Ser Leu  
 1810 1815 1820  
 Glu Ile Leu Glu Gly Leu Gly Leu Ser Glu Arg Gly Leu Asn Ala Ser  
 1825 1830 1835 1840  
 Asn Gly Leu Asn Gly Leu Asn Gly Leu Leu Tyr Ser Ala Ser Asn Gly  
 1845 1850 1855  
 Leu Gly Leu Asn Gly Leu Leu Glu Leu Glu Gly Leu Leu Glu Ala Ser  
 1860 1865 1870  
 Pro Leu Tyr Ser Thr Arg Pro Ala Leu Ala Ser Glu Arg Leu Glu Thr  
 1875 1880 1885

Arg Pro Ser Glu Arg Thr Arg Pro Pro His Glu Ala Ser Pro Ile Leu  
 1890 1895 1900

Glu Ser Glu Arg Ala Ser Asn Thr Arg Pro Leu Glu Thr Arg Pro Thr  
 1905 1910 1915 1920

Tyr Arg Ile Leu Glu Leu Tyr Ser Ile Leu Glu Pro His Glu Ile Leu  
 1925 1930 1935

Glu Met Glu Thr Ile Leu Glu Val Ala Leu Ala Leu Ala Gly Leu Tyr  
 1940 1945 1950

Leu Glu Val Ala Leu Gly Leu Tyr Leu Glu Ala Arg Gly Ile Leu Glu  
 1955 1960 1965

Val Ala Leu Pro His Glu Ala Leu Ala Val Ala Leu Leu Glu Ser Glu  
 1970 1975 1980

Arg Ile Leu Glu Val Ala Leu Ala Ser Asn Ala Arg Gly Val Ala Leu  
 1985 1990 1995 2000

Ala Arg Gly Gly Leu Asn Gly Leu Tyr Thr Tyr Arg Ser Glu Arg Pro  
 2005 2010 2015

Arg Leu Glu Ser Glu Arg Pro His Glu Gly Leu Asn Thr His Arg His  
 2020 2025 2030

Ile Ser Pro His Glu Pro Arg Ala Leu Ala Pro Arg Ala Arg Gly Gly  
 2035 2040 2045

Leu Tyr Pro Arg Ala Ser Pro Ala Arg Gly Pro Arg Ala Ser Pro Gly  
 2050 2055 2060

Leu Tyr Ile Leu Glu Gly Leu Gly Leu Tyr Gly Leu Gly Leu Tyr Gly  
 2065 2070 2075 2080

Leu Tyr Gly Leu Ala Arg Gly Ala Ser Pro Ala Arg Gly Ala Ser Pro  
 2085 2090 2095

Ala Arg Gly Ser Glu Arg Val Ala Leu Ala Arg Gly Leu Glu Val Ala  
 2100 2105 2110

Leu Ala Ser Pro Gly Leu Tyr Pro His Glu Leu Glu Ala Leu Ala Leu  
 2115 2120 2125

Glu Leu Glu Thr Arg Pro Gly Leu Ala Ser Pro Leu Glu Ala Arg Gly  
 2130 2135 2140

Ala Ser Asn Leu Glu Cys Tyr Ser Leu Glu Pro His Glu Ser Glu Arg  
 2145 2150 2155 2160

Thr Tyr Arg His Ile Ser Ala Arg Gly Leu Glu Ala Arg Gly Ala Ser  
 2165 2170 2175

Pro Leu Glu Leu Glu Leu Glu Ile Leu Glu Val Ala Leu Thr His Arg  
 2180 2185 2190

Ala Arg Gly Ile Leu Glu Val Ala Leu Gly Leu Leu Glu Leu Glu Gly  
 2195 2200 2205  
 Leu Tyr Ala Arg Gly Ala Arg Gly Gly Leu Tyr Thr Arg Pro Gly Leu  
 2210 2215 2220  
 Ala Leu Ala Leu Glu Leu Tyr Ser Thr Tyr Arg Thr Arg Pro Thr Arg  
 2225 2230 2235 2240  
 Pro Ser Glu Arg Leu Glu Leu Glu Gly Leu Asn Thr Tyr Arg Thr Arg  
 2245 2250 2255  
 Pro Ser Glu Arg Gly Leu Asn Gly Leu Leu Glu Leu Tyr Ser Ala Ser  
 2260 2265 2270  
 Asn Ser Glu Arg Ala Leu Ala Val Ala Leu Ala Ser Asn Leu Glu Pro  
 2275 2280 2285  
 His Glu Ala Ser Asn Thr His Arg Ala Arg Gly Ala Leu Ala Ile Leu  
 2290 2295 2300  
 Glu Val Ala Leu Val Ala Leu Ala Leu Ala Gly Leu Gly Leu Tyr Thr  
 2305 2310 2315 2320  
 His Arg Ala Ser Pro Ala Arg Gly Ile Leu Glu Ile Leu Glu Gly Leu  
 2325 2330 2335  
 Val Ala Leu Val Ala Leu Gly Leu Asn Ala Arg Gly Leu Glu Cys Tyr  
 2340 2345 2350  
 Ser Ala Arg Gly Ala Leu Ala Ile Leu Glu Leu Glu His Ile Ser Ile  
 2355 2360 2365  
 Leu Glu Pro Arg Ala Arg Gly Ala Arg Gly Ile Leu Glu Ala Arg Gly  
 2370 2375 2380  
 Gly Leu Asn Gly Leu Tyr Leu Glu Gly Leu Ala Arg Gly Pro His Glu  
 2385 2390 2395 2400  
 Leu Glu Leu Glu

We claim:

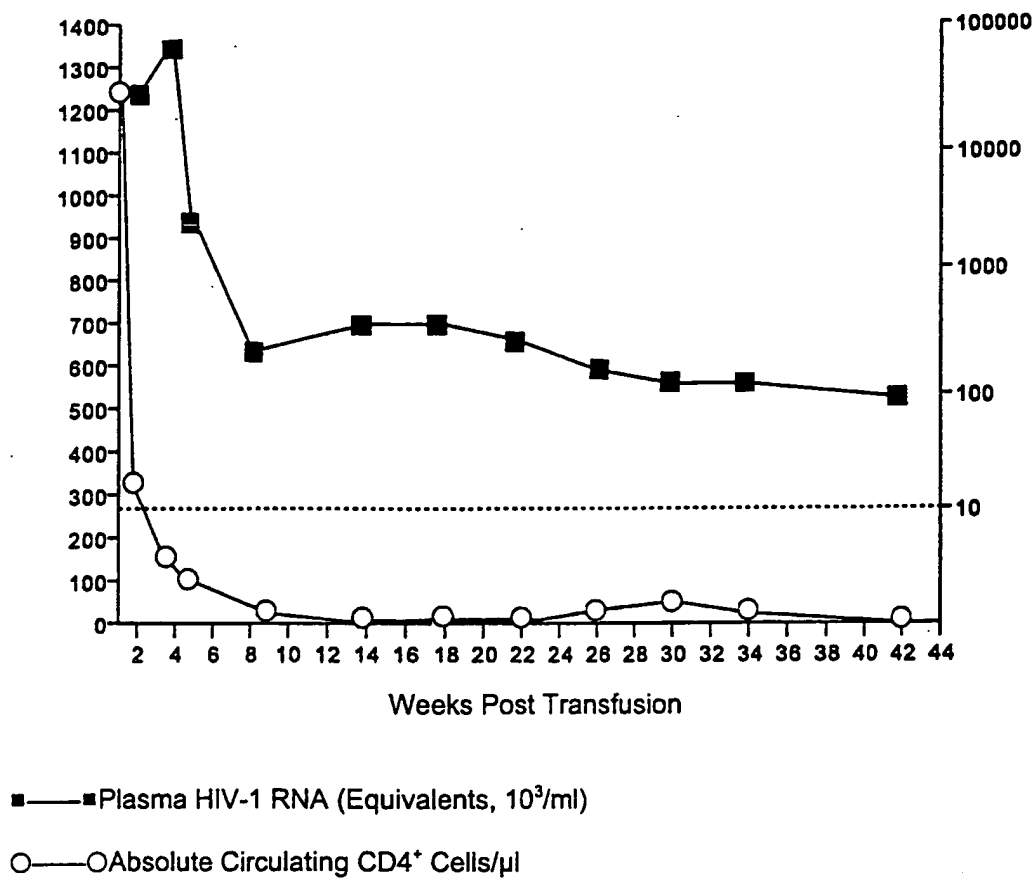
1. An isolated human immunodeficiency virus type 1 (HIV-1), which is HIV-1<sub>JC</sub>, identified by a nucleotide sequence as given in SEQ ID NO:11, or which is HIV-1<sub>NC</sub>, identified by a nucleotide sequence as given in SEQ ID NO:12.
2. A biological sample comprising the HIV-1<sub>JC</sub> of claim 1.
3. A biological sample comprising the HIV-1<sub>NC</sub> of claim 1.
4. The biological sample of claim 1 wherein said sample is a sample of blood.
5. A biologically pure culture of host cells comprising the HIV-1<sub>JC</sub> or HIV-1<sub>NC</sub> of claim 1.
6. The culture of claim 5 wherein the host cells are peripheral blood mononuclear cells.
7. A composition comprising an antigenic preparation of the HIV-1<sub>JC</sub> of claim 1.
8. A composition comprising an antigenic preparation of the HIV-1<sub>NC</sub> of claim 1.
9. A kit for detecting the presence of HIV-1 antibodies comprising an antigenic preparation of the HIV-1<sub>JC</sub> or HIV-1<sub>NC</sub> of claim 1.
10. An immunogenic composition comprising an antigenic preparation of the HIV-1<sub>JC</sub> or HIV-1<sub>NC</sub> of claim 1 and a pharmaceutically acceptable carrier.
11. A vaccine comprising an antigenic preparation of the HIV-1<sub>JC</sub> or HIV-1<sub>NC</sub> of claim 1.
12. An isolated DNA molecule comprising a nucleotide sequence encoding an infectious molecular clone for HIV-1<sub>JC</sub> or an antigenic fragment thereof or for HIV-1<sub>NC</sub> or an antigenic fragment thereof.

13. The isolated DNA molecule of claim 12, wherein the DNA comprises a nucleotide sequence encoding an HIV-1<sub>JC</sub> envelope protein as given in SEQ ID NO:2, an HIV-1<sub>JC</sub> gag, nef or p24 protein, or an antigenic fragment of any of the foregoing..
14. The isolated DNA molecule of claim 13, wherein the DNA comprises the nucleotide sequence of SEQ ID NO:1 or a portion thereof specifying an antigenic fragment of said HIV-1<sub>JC</sub> envelope protein.
15. The isolated DNA molecule of claim 12, wherein the DNA comprises a nucleotide sequence encoding an HIV-1<sub>NC</sub> envelope protein as given in SEQ ID NO:23, an HIV-1<sub>NC</sub> gag, nef or p24 protein, or an antigenic fragment of any of the foregoing.
16. A method of inducing antibodies to HIV-1<sub>JC</sub> in a nonhuman mammalian subject comprising the steps of (a) administering to the subject an immunogenic amount of an antigenic preparation of the HIV-1<sub>JC</sub> and optionally (b) harvesting said antibodies to HIV-1<sub>JC</sub>.
17. The method of claim 16 wherein said subject is a primate.
18. A method of inducing antibodies to HIV-1<sub>NC</sub> in a nonhuman mammalian subject comprising the steps of (a) administering to the subject an immunogenic amount of an antigenic preparation of the HIV-1<sub>NC</sub> and optionally (b) harvesting said antibodies to HIV-1<sub>NC</sub>.
19. The method of claim 18 wherein said subject is a primate.
20. A method of immunizing a subject against the development of acquired immune deficiency syndrome (AIDS) comprising the step of administering to said subject an immunogenic amount of an antigenic preparation of HIV-1<sub>JC</sub> or HIV-1<sub>NC</sub> such that antibodies directed to HIV-1<sub>JC</sub> or HIV-1<sub>NC</sub> are produced in said subject and thereby symptoms of AIDS are diminished or prevented.
21. The method of claim 20 wherein said subject is a primate.

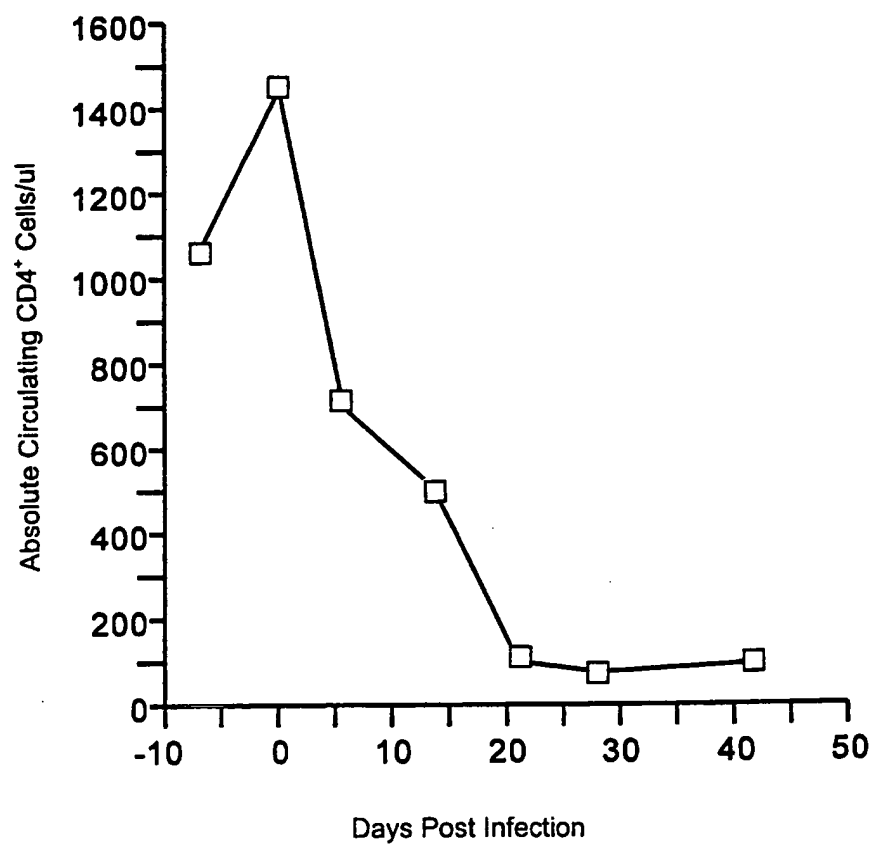


22. A method for inducing acquired immune deficiency syndrome (AIDS) in a nonhuman primate, comprising the step of administering to said primate an effective amount of an infections preparation of the HIV-1 of claim 1 such that said primate develops AIDS.
23. The method of claim 22 wherein said infections preparation of HIV-1 is a biological specimen obtained from a nonhuman primate infected with HIV-1<sub>IC</sub> and exhibiting AIDS.
24. The method of claim 22 wherein said infections preparation of HIV-1 is a biological specimen obtained from a nonhuman primate infected with HIV-1<sub>NC</sub> and exhibiting AIDS.
25. A method of screening for HIV-1 in a biological sample comprising the step of introducing into said biological sample a hybridization probe comprising a nucleotide sequence comprising at least about 15 contiguous nucleotides from SEQ ID NO:11 or a sequence complementary thereto or from SEQ ID NO:12 or a nucleotide sequence complementary thereto under stringent conditions such that said hybridization probe binds to an HIV-1<sub>IC</sub> gene or a nucleotide sequence having at least about 95% nucleotide sequence identity thereto or an HIV-1<sub>NC</sub> gene or a nucleotide sequence having at least about 95% nucleotide sequence identity thereto.
26. A nonhuman primate model infected with at least one HIV-1 of claim 1 and exhibiting symptoms of AIDS, useful for the development of a drug or vaccine for the treatment or prevention of AIDS.
27. The primate model of claim 26 wherein said primate is a chimpanzee or a macaque.
28. The primate model of claim 27 wherein said HIV-1 is HIV-1<sub>IC</sub>.
29. The primate model of claim 27 wherein said HIV-1 is HIV-1<sub>NC</sub>.

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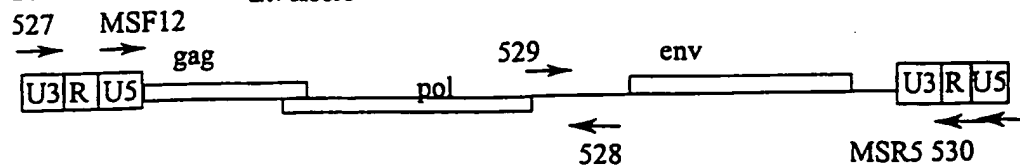
**FIG. 1**

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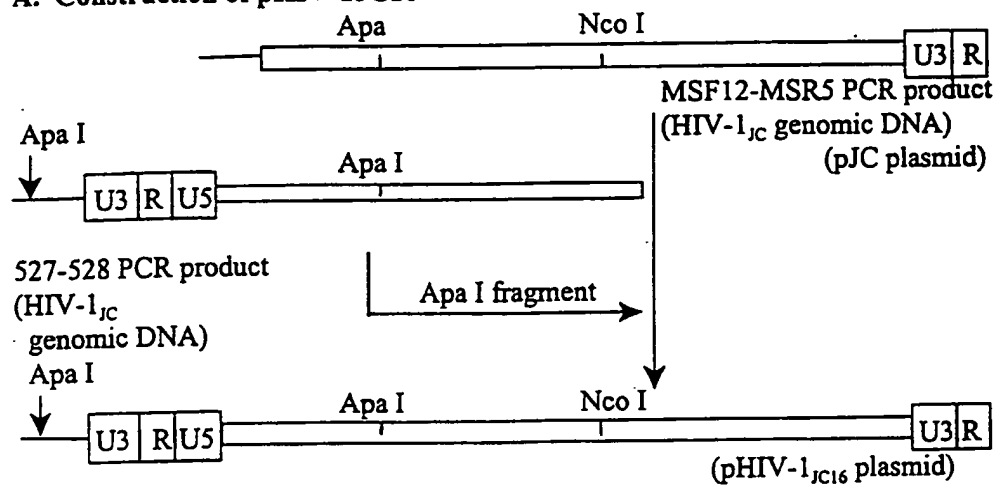
**FIG. 2**

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Structure of HIV-1<sub>LAV and SF2</sub>



A. Construction of pHIV-1<sub>JC16</sub>



B. Construction of pHIV-1<sub>NC7</sub>

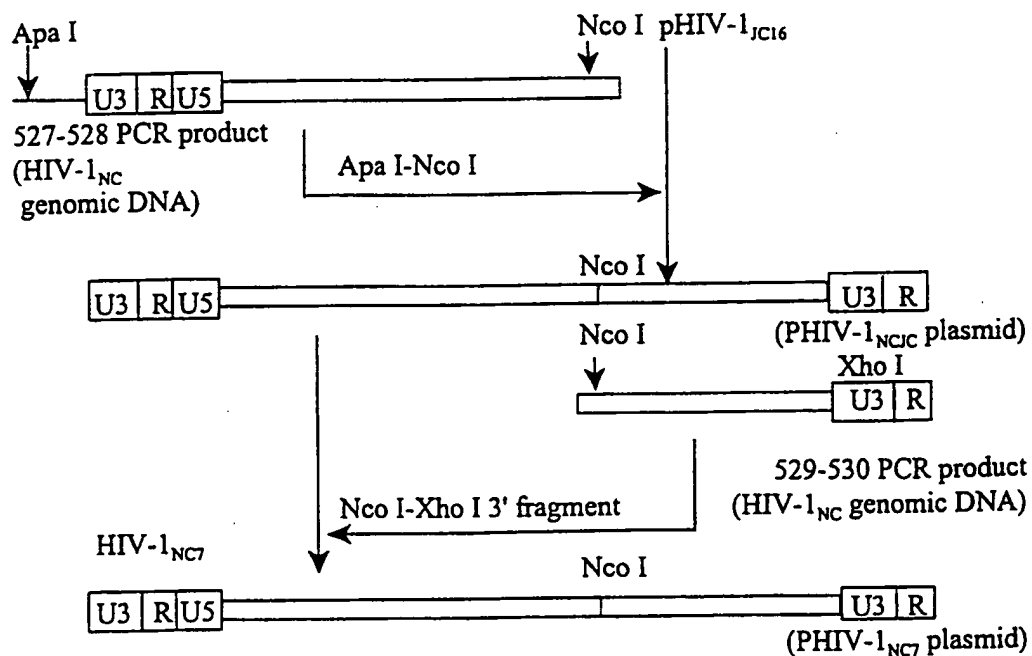
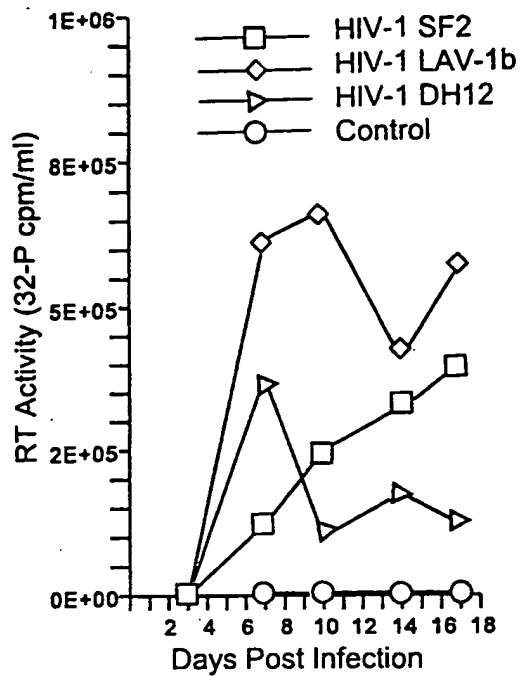
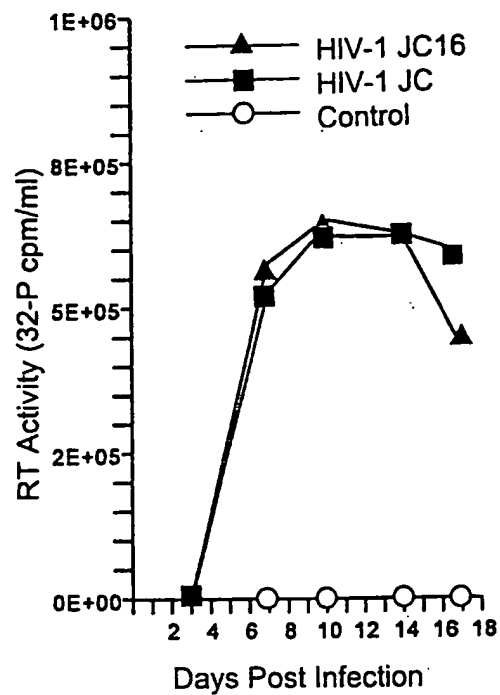
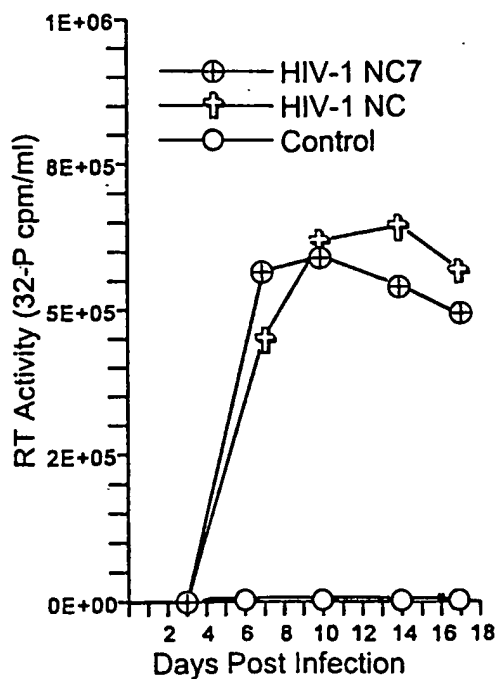
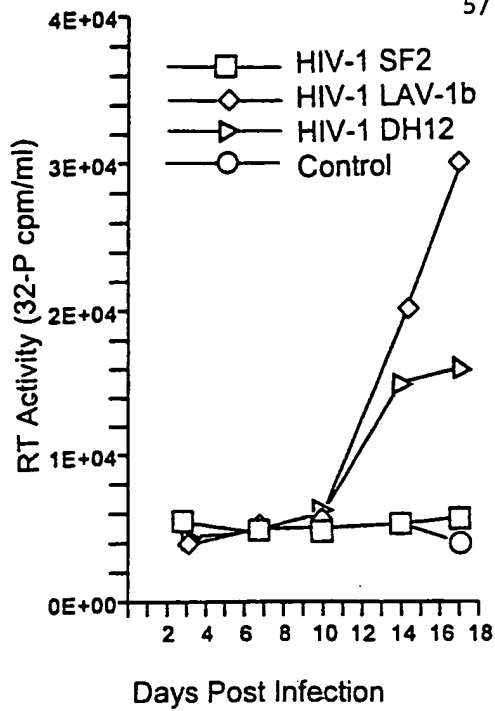


FIG. 3

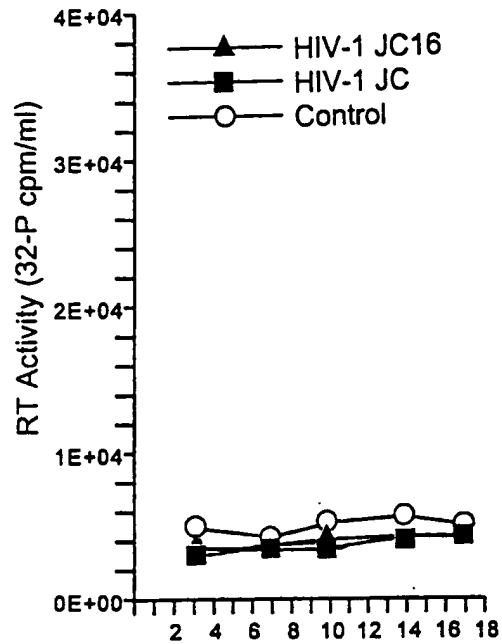
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**FIG. 4A****FIG. 4B****FIG. 4C**

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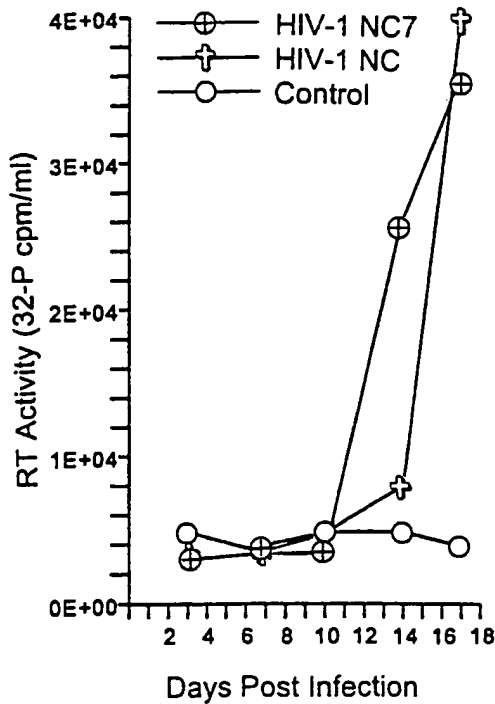


**FIG. 4D**



Days Post Infection

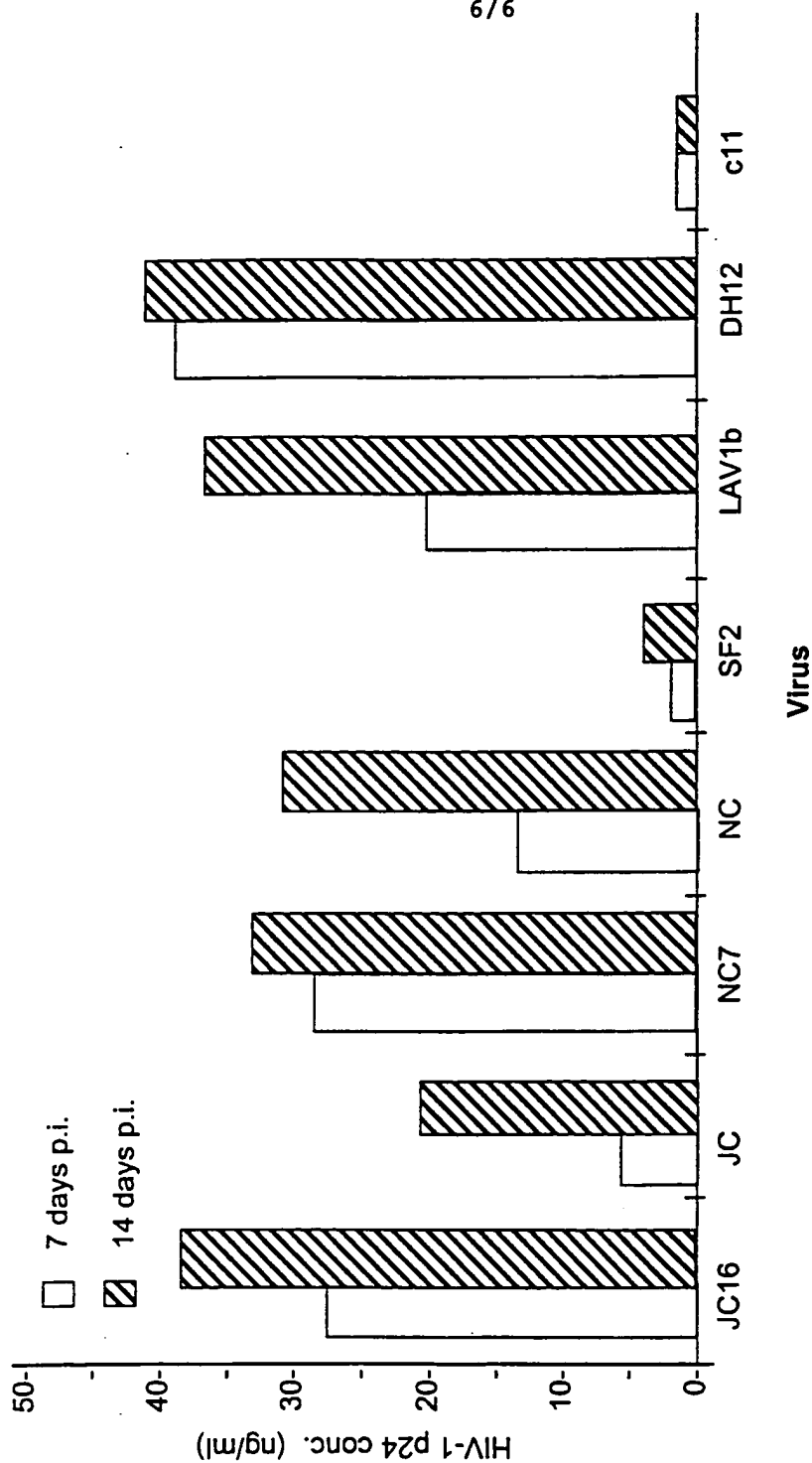
**FIG. 4E**



Days Post Infection

**FIG. 4F**

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**FIG. 5**

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/12990**A. CLASSIFICATION OF SUBJECT MATTER**IPC(6) : C12Q 1/68, 1/70; C12N 7/00, 7/01, 7/02; G01N 33/564; C07K 1/00  
US CL : 435/5, 6, 235, 239, 974; 530/350, 826

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/5, 6, 235, 239, 974; 530/350, 826

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline, Aidsline

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NOVEMBRE et al Development of AIDS in a Chimpanzee Infected with Human Immunodeficiency Virus Type 1 Journal Of Virology. May 1997, Vol. 71, No. 5, pages 4086-4091, see entire document.	1-15
X	VINCENT et al. Characterization of a Novel Baboon Virus Closely Resembling Human T-Cell Leukemia Virus Virology. 01 December 1996, Vol. 226, No. 1, pages 57-65, see entire document.	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

20 AUGUST 1998

Date of mailing of the international search report

18 SEP 1998

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
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**INTERNATIONAL SEARCH REPORT**International application No.  
PCT/US98/12990**Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:  
1-15 only to the extent of the elected specie, envelope protein.
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/12990

### BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-12, drawn to a virus.

Group II, claims 13-15, drawn to viral proteins.

Group III, claims 16-21, drawn to a method of inducing antibodies.

Group IV, claims 22-4 and 26-29, drawn to a primate model.

Group V, claim 25, drawn to a method of screening for HIV infection.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The rules of PCT unity of invention do not allow for multiple inventions. Each of the inventions requires a different search and a search of each of the inventions would not be co-extensive with each of the other searches.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

Group II: envelope, gag, nef, and p24.

The claims are deemed to correspond to the species listed above in the following manner:

Group II, claims 13-15

The following claims are generic: 1-12.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Each of the proteins is a different compound and has different characteristics such as biological function and immunological reactivity.